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(54) Title: ENVELOPE ANTIGENS OF LYMPHADENOPATHY ASSOCIATED VIRUS AND THEIR APPLICATIONS

(57) Abstract

Purified expression products of DNA sequences derived of the genome of the LAV virus. It relates more particularly to a glycoprotein having a molecular weight of about 110,000 or antigen of lower molecular weight derived from the preceding one, which purified product possesses the capacity of being recognised by serums of human origin and containing antibodies against the LAV virus. It also relates to other purified polypeptides derived either from the abovesaid glycoprotein or of core proteins of LAV virus. The glycoprotein can be used for the production of immunogenic composition capable of neutralizing the LAV virus. All of the abovesaid glycoprotein or polypeptides can be used as antigens useful in the diagnosis of LAV antibodies in sera of patients.

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Envelope antigens of lymphadenopathy-associated virus and their applications.

The present invention relates to antigens, particularly in a purified form, of the virus of lymphadenopathies (denoted below by the abbreviation LAS) and of the acquired immuno-depressive syndrome (denoted below by the abbreviation AIDS), to a process for producing these antigens, particularly antigens of the envelopes of these viruses. The invention also relates to polypeptides, whether glycosylated or not, encoded by said DNA sequences.

The causative agent if LAS or AIDS, a retrovirus, has been identified by F. BARRE-SINOUSSI et al, Science, 220, 868 (1983). It has the following characteristics. It is T-lymphotropic; its prefered target is constituted by Leu 3 cells (or T4 lymphocytes) ; it has reverse transcriptase activity necessitating the presence of Mg + and exhibits strong affinity for poly(adenylate-oligodeoxy-thymidylate)(poly(A)-oligo(dT)12-18). It has a density of 1.16-1.17 in a sucrose gradient, an average diameter of 139 nanometers; and a nucleus having an average diameter of 41 nanometers. Antigens of said virus, particularly a protein p25 are recognised immunologically by antibodies contained in serums taken up from patients afflicted with LAS or AIDS. The p25 protein, which is a core protein, is not recognised immunologically by the p24 protein of the HTLVI and II viruses. The virus is also free of a p19 protein which is immunologically cross-reactive with the p19 proteins of HTLVI and HTLVII.

Retroviruses of this type (sometimes denoted by the generic abbreviation LAV) have been filed in the National Collection of Micro-organism Cultures of the INSTITUT PASTEUR of Paris, under numbers I-232, I-240 and I-241. Virus strains similar to LAV in all respects from the morphological and immunological point of view have been isolated in other laboratories. Reference is made by way of examples to the retrovirus strains named HTLV-III isolated by R.C. GALLO et al., Science, 224, 500 (1984) and by M.G. SARNGADHARAN et al., Science 224, 506 (1984) respectively and to the retrovirus isolated by M. JAY LEVY et al., Science, 225, 840-842 (1984), which virus was designated ARV. For the ease of language the last mentioned viruses, as well as others which have equivalent morphological and immunological properties, will be designated hereafter under the generic designation "LAV". Reference is also made to European patent application filed 14 September 1984, with the priority of British patent application number 83 24800 filed 15 september 1983 as regards a more detailed description of the LAV retroviruses or the like and of the uses to which extracts of these viruses give rise.

Initially the core antigens were the main antigens of the virus lysates or extracts which were recognised by serums of patients infected with AIDS or LAS, in the test systems which had then been used. A p42 protein, presented as consisting of an envelope protein, had been detected too. In the same manner GALLO et al disclosed a p41 protein which was also deemed to be on a possible component of the viru envelope.

Processes for obtaining a LAV virus have also been described. Reference may be made particularly to the article already mentioned of F. BARRE-SINOUSSI et al., as regards the preparation of the virus in T lymphocyte cultures derived either from blood, or from the umbilical cord, or also from bone marrow cells of adult

donors in good health. This process comprises particularly the following essential steps :

- producing a viral infection of these T lymphocytes, after activation by a lectin mitogen, with a viral suspension derived from a crude supernatant liquor of lymphocytes producing the virus (initially obtained from a patient infected with AIDS or LAS),
- culturing cells infected with TCGF, in the presence of anti-α-interferon sheep serum,
- effecting purification of the virus produced (production starts generally between the 9th and the 15th day following infection and lasts from 10 to 15 days), which purification comprises precipitating the virus in polyethylenglycol in order to produce a first concentration of the virus, then centrifugating the preparation obtained in a 20-60 % sucrose gradient or in an isotonic gradient of metrizanide (sold under the trade mark NYCODENZ by NYEGAARD, Oslo) and recovering the virus with the band having a density of 1.16-1.17 in the sucrose gradient or of 1.10-1.11 in the NYCODENZ gradient.

The LAV virus may also be produced from permanent cell lines of type T, such as the CEM line, or from B lymphoblastoid cell lines, such as obtained by the transformation of the lymphocytes derived from a healthy donor with the Epstein-Barr virus, for instance as disclosed in French patent application Nr. 84 07151 filed May 9, 1984. The permanent cell lines obtained produce continuously a virus (designated as LAV-B in the case of the B lymphoblastoid cell lines) which possesses the essential antigenic and morphological features of the LAV viruses (except that it is collected in a density band sometimes slightly higher than in the preceding case (particularly 1.18) in sucrose. The final purification of the virus can also be carried out in a NYCODENZ gradient.

A method for cloning DNA sequences hybridizable with the genomic RNA of LAS has already been disclosed in British Patent Application Nr. 84 23659 filed on September 19, 1984. Reference is hereafter made to that application as concerns subject matter in common with the further improvements to the invention disclosed herein.

The invention aims at providing purified unaltered virus forms (or viruses less altered by the purification procedures resorted to) and processes for obtaining said unaltered purified viruses.

The present invention further aims at providing additional new means which should not only also be useful for the detection of LAV or related viruses (hereafter more generally referred to as "LAV viruses"), but also have more versatility, particularly in detecting specific parts of the genomic DNA of said viruses whose expression products are not always directly detectable by immunological methods. The present invention further aims at providing polypeptides containing sequences in common with polypeptides comprising antigenic determinants included in the proteins encoded and expressed by the LAV genome occuring in nature. An additional object of the invention is to further provide means for the detection of proteins related to LAV virus, particularly for the diagnosis of AIDS or pre-AIDS or, to the contrary, for the detection of antibodies against the LAV virus or proteins related therewith, particularly in patients afflicted with AIDS or pre-AIDS or more generally in asymtomatic carriers and in blood-related products. Finally the invention also aims at providing immunogenic polypeptides, and more particularly protective polypeptides for use in the preparation of vaccine compositions against AIDS or related syndroms.

The present invention relates to additional DNA fragments, hybridizable with the genomic RNA of LAV as they will be disclosed hereafter, as well as with additional cDNA variants corresponding to the whole genomes of LAV viruses. It further relates to DNA recombinants containing said DNAs or cDNA fragments.

An unaltered purified LAV retrovirus distinguishes from those which have been defined above, in that it includes an amount of one or several envelope antigens, sufficient to be visualized when the virus is labelled with ^{35}S-cystein, free of unlabelled cystein in a proportion of 200 microcuries per ml of medium. these antigens, among which particularly glycoproteins, are recognised selectively in vitro by serums of patients affected with SIDA or SLAs or by the serums of asymptomatic carriers of the virus.

A preferred antigen according to the preceding definition obtainable from a lysate of this virus (or by gentle scouring of the envelopes of the virus) is a glycoprotein having a molecular weight of the order of 110,000 daltons, as determined by its migration distance in comparison with the distances of migrations, in a same migration system, of standard proteins having known molecular weights. Particularly comparative measurements were made on a 12.5 % polyacrylamid gel under a voltage of 18 V for 18 hours, upon using the following standard proteins (marketed by AMERSHAM) :

- lysozyme-(^{14}C)-methyl (MW: 14,300),
- carbon dioxide-(^{14}C)-methyl (MW: 30,000),
- ovalbumin-(^{14}C)-methyl (MW: 46,000),
- bovin albumin serum (^{14}C)-methyl (MW: 69,000),
- phosphorylase b-(^{14}C)-methyl (MW: 92,500),
- myosine-(^{14}C)-methyl (MW: 200,000).

The invention relates also to the antigens themselves, particularly that of molecular weight of about 110,000-120,000, which possess also the capability

of being recognised by serums of patients infected with AIDS or LAS or by serums of persons who have been exposed to LAV viruses or those analogous with the latter. These antigens have also the characteristic of forming complexes with concanavaline A, said complex being dissociatable in the presence of O-methyl-α-D-mannopyranoside. The antigens according to the invention can also bind to other lectins for example those known under the name "LENTYL-LECTIN". The preferred antigen according to the invention, of molecular weight 110,000, is also sensitive to the action of endoglycosidases. This action is manifested by the production from the antigen of molecular weight 110,000 of a protein having a molecular weight of the order of 90,000, the latter being separable for example by immunoprecipitation or by separation employing the differences in molecular weights (migrations differentiated on gel).

Preferred antigens of the invention are constituted by glycoproteins.

The invention relates also to the process for producing the viruses according to the invention. This process distinguishes essentially from those recalled above at the level of the final purification operation. In particular, the purification step of the process according to the invention is no longer carried out in gradients, but involves the performance of differential centrifugations effected directly on the supernatants of the culture media of the producing cells. These centrifugation operations comprise particularly a first centrifugation at an angular centrifugation velocity, particularly of 10,000 rpm, enabling the removal of non-viral constituents, more particularly of cellular constituents, then a second centrifugation at higher angular velocity, particularly at 45,000 rpm, to obtain the precipitation of the virus itself. In preferred embodiments, the first centrifugation at 10,000 rpm, is

maintained for 10 minutes and the second at 45,000 rpm, for 20 minutes. These are, of course, only indicative values, it being understood that it remains within the ability of the specialist to modify the centrifugation conditions, to provide for the separation of the cellular constituents and of the viral constituents.

This modification of the purification process results in the production of viral preparaations from which the antigen mentioned can then be isolated more easely, than from virus preparations purified by the previous methods. In any event, the viruses finally obtained by the process of the present invention are more easely recognised by serums of patients or of persons who have been exposed to the LAV virus or to morphologically and antigenically similar strains.

The antigens according to the invention can themselve be obtained from the above disclosed viruses, by lysis (or other suitable processing) of the latter in the presence of any suitable detergent and by recovery and separation of the antigens released. Advantageously, the lysis of the virus is effected in the presence of aprotinin or of any other agent suitable for inhibiting the action of proteases. The separation of the antigens according to the invention can then be carried out by any method known in itself ; for example, it is possible to proceed with a separation of the proteins by employing their respectively different migrations in a predetermined gel, the protein sought being then isolated from the zone of the gel in which it would normally be found in an electrophoresis operation under well determined conditions, having regard to its molecular weight. The antigens according to the invention can however be separated from the lysate of the abovesaid viruses, due to their affinity for lectins, in particular concanavaline A or lentyl-lectin. The lectin used is preferably immobilised on a solid support, such

as the cross linked polymer derived from agarose and marketed under the trade mark SEPHAROSE. After washing of the fixed antigens with a suitable buffer, the antigens can be eluted in any suitable manner, particularly by resorting to a O-methyl-α-D-mannopyranoside in solution.

A more thorough purification of these antigens can be performed by immunoprecipitation with the serums of patients known to possess antibodies effective against said protein, with concentrated antibody preparations (polyclonal antibodies) or again with monoclonal antibodies, more particularly directed against the antigen according to the invention, in particular that having the molecular weight of 110,000, denoted below by the abbreviation gp110.

Additional characteristics of the invention will appear also in the course of the description which follows of the isolation of a virus according to the invention and of antigens, particularly an envelope antigen of the virus. reference will be made to the drawings in which :

Figure 1 is derived from a photographic reproduction of gel strips which have been used to carry out electrophoreses of lysate extracts of T lymphocytes, respectively infected and uninfected (controls) by a LAV suspension.

Figure 2 is the restriction map of a complete LAV genome (clone λJ19).

Figures 3a to 3e are the complete sequence of a LAV viral genome.

Figures 4 and 5 show diagrammatically parts of the three possible reading phases of LAV genomic RNA, including the open reading frames (ORF) apparent in each of said reading phases.

Figure 6 is a schematic representation of the LAV long terminal repeat (LTR).

I - PRODUCTION OF THE VIRUS AND OF ANTIGENS

T lymphocytes derived from a healthy donor and infected with LAV1, under the conditions described by F. BARRE-SINOUSSI et Coll., on CEM cells derived from a patient afflicted with leukemia and also infected in vitro with LAV1, were kept under cultivation in a medium containing 200 microcuries of ^{35}S-cystein and devoid of unlabelled cystein. The infected lymphocytes were cultured in a non denaturating medium to prevent the degradation of the antigen sought. The supernatant liquor from the culture medium was then subjected to a first centrifugation at 10,000 rpm for 10 minutes to remove the non viral components, then to a second centrifugation at 45,000 rpm for 20 minutes for sedimenting the virus. the virus pellet was then lysed by detergent in the presence of aprotinin (5 %) particularly under the conditions described in the article of F. BARRE-SINOUSSI et Coll.

The same operation was repeated on lymphocytes taken up from a healthy donor as control.

The various lysates were then immuno-precipitated by serums of patients infected with AIDS or with LAS. Serums originating from healthy donors or of donors infected with other diseases were immunoprecipitated too. The media were then subjected to electrophoreses in a SDS-polyacrylamide gel.

The results are indicated in figure 1. The gel strips numbered from 1 to 6 were obtained from preparations labelled by ^{35}S-cystein. The strips numbered 7 to 10 show results observed on infected or uninfected lymphocyte preparations labelled with ^{35}S-methionine. Finally the strip M corresponds to the migration distances of the standard proteins identified above, whose

molecular weights are recalled in the right hand portion of the figure.

The references to the labelled viral proteins appear on the left handside of the figure.

It is noted that columns 7 to 10 show the specific protein p25 of LAV, labelled with ^{35}S-methionin. The same protein is absent on strips 8 to 10 corresponding to results obtained with a preparation originating from healthy lymphocytes.

Columns 3 and 5 correspond to the results which have been observed on preparations obtained from lymphocytes infected and labelled with ^{35}S-cystein. The proteins p25 and p18, the characteristic core proteins of LAV, and the glycoprotein gp110, also specific of LAV, were also present. Images corresponding to a protein p41 (molecular weight of the order of 41,000) appeared in the various preparations, although less distinctly.

The virus according to the invention and the antigen according to the invention can be either precipitated by lectins, particularly concanavaline A, or fixed to a SEPHAROSE-concanavaline A column. Particularly the purification of the envelope glycoproteins can be carried out as follows. This fixation can particularly be carried out by contacting a lysate of the LAV virus dissolved in a suitable buffer with concanavaline-A bound to SEPHAROSE. A suitable buffer has the following composition :

Tris	10 mM
NaCl	0.15 M
$CaCl_2$	1 mM
$MgCl_2$	1 mM

Detergent marketed under the trade mark TRITON 1 %

pH	7.4

When the fixation has been achieved, the SEPHAROSE-concanavaline A is washed with a buffer of the

same composition, except that the TRITON concentration is lowered to 0.1 %. The elution is then effected with an 0.2 M O-methyl-α-D-mannopyranoside solution in the washing buffer.

The protein may be further concentrated by immuno-precipitation with antibodies contained in the serums of patients infected with AIDS or with polyclonal antibodies obtained from a serum derived from an animal previously immunised against the "unaltered" virus according to the invention or the abovesaid glycoprotein. The protein can then be recovered by dissociation of the complex by a solution having an adequate content of ionic salt. Preferably the antibody preparation is itself immobilised in a manner known in itself on an insoluble support, for instance of the SEPHAROSE B type.

It is also possible to resort to monoclonal antibodies secreted by hybridomas previously prepared against gp 110. These monoclonal antibodies, as well as the hybridomas which produce them, also form part of the invention.

A technique for producing and selecting monoclonal antibodies directed against the gp110 glycoprotein is described below.

Immunisation of the mice

Groups of Balb/c mice from 6 to 8 weeks old mice were used. One group receives the virus carrying the abovesaid glycoprotein, another a purified glycoprotein gp110. The immunisation procedure, identical for all mice, comprises injecting 10 mg of the antigenic preparation in the prsence of Freund complete adjuvant at day 0, then again but in the presence of Freund incomplete adjuvant at day 14 and without adjuvant at days 28 and 42. The three first injections are made intraperitoneally, the fourth intravenously.

Fusion and culture of the hybrids

The non secreting myeloma variant 5.53 P3 x 63 Ag8, resistant to azaguanine, itself derived from the MOPC-21 cell-line, is used. Fusion with immunised mouse splenocytes is carried out in the presence of polyethylene-glycol 4000 by the technique of FAZEKAS de st-GROTH and SCHEIDEGGER on the 45th day. The selection of the hybrids in RPMI 16-40 "HAT" medium is carried out in plates having 24 cups (known under the designation COSTAR) by resorting to the same culture techniques.

The hybridomas producing antibodies of adequate specificity are then cloned in plates having 96 cups, in the presence of a "feeder" layer of syngenic thymocytes. The producing clones thus selected are then expanded in 24 cup plates, still in the presence of thymocytes. When the confluence appears in one of the cups, the clone is injected intraperitoneally into a balb/c mouse which had received an injection of PRISTANE 8 days previously and/or kept in liquid culture.

Demonstration of the anti-LAV antibodies

Five different techniques enable characterisation of the clones producing antibodies of suitable specificity. In a first stage, the hybrids producing antibodies are determined by an ELISA test revealing mouse immunoglobulins in the supernatant liquors. From this first selection, supernatants are sought which have antibodies directed against viral constituents by means of an ELISA test revealing anti-LAV antibodies, or by immunofluorescence on the virus producing human cells. Finally the supernatant liquours are analysed by radioimmunoprecipitation of virus labelled with cystein and by the Western-Blot technique on viral preparation which permit the determination of the specificities of these anti-LAV antibodies.

RESULTS

Cells obtained from the various fusions are placed under culture in 648 cups. Their microscopic examination shows that the majority of these cups contain a single hybrid clone capable of growing in a "HAT" selective medium. More than 50 % among them produce antibodies giving rise to a positive response under ELISA antivirus examination. The most representative fusions are tested by the Western-Blot technique and several of them are subcloned, taking into account their respective specificities reactivities in antivirus ELISA and their behaviours under the culturing conditions. Those hybrids which are more particularly selected are those which produce antibodies which selectively recognise the viral glycoprotein gp110 having a molecular weight of about 110 KD. All the sub clonings give rise to clones producing antibodies which, after expression, are injected into syngenic mice. Analysis of the specificities of the antibodies present in the different ascites liquids confirm the specificity of the antibodies of said ascites with respect to gp110.

The monoclonal antibodies obtained can themselves be employed to purify proteins containing an antigenic site also contained in gp110. The invention relates therefore also to these processes of purification as such. This process is advantageously applied to virus lysates or T lymphocyte lysates or other cells producing LAV or the like, when care has been taken to avoid the uncontrolled separation of gp110 during the purification procedure of the virus, prior to lysys thereof. Needless to say that the process can also be applied to any solution containing gp110 or a protein, polypeptide or glycoprotein comprising an antigenic site normally carried by the envelope protein and recognised by the monoclonal antibody. For practising this process, the monoclonal antibodies are advantageously immobilised

on a solid support, preferably adapted to affinity chromatography operations. For example, these monoclonal antibodies are fixed to an agarose lattice with three-dimensional cross-linking, marketed under the trade mark SEPHAROSE by the Swedish company PHARMACIA A.G., for example by the cyanogen bromide method.

The invention therefore also relates to a process for separating the antigens concerned, which process comprises contacting a mixture of antigens, including those of interest (for instance a virus lysate or extract), with an affinity column bearing the above-said monoclonal antibodies, to selectively fix polypeptides, proteins or glycoproteins selectively recognized by said monoclonal antibodies, recovering the latter by dissociation of the antigen-antibody complex by means of a suitable buffer, particularly a solution of adequate ionic strength, for example of a salt, preferably ammonium acetate (which leaves no residue upon freeze drying of the preparation or a solution acidified to a pH 2-4 or to a glycine buffer at the same pH and recovering the eluted polypeptides, proteins or glycoproteins.

It is self-evident that the invention relates also to polypeptide fragments having lower molecular weights and carrying antigenic sites recognizable by the same monoclonal antibodies. It is clear to the specialist that the availabililty of monoclonal antibodies recognizing the gp110 glycoprotein gives also access to smaller peptide sequences or fragments containing the common antigenic site or epitope. Fragments of smaller sizes may be obtained by resorting to known techniques. For instance such a method comprises cleaving the original larger polypeptide by enzymes capable of cleaving it at specific sites. By way of examples of such proteins, may be mentioned the enzyme of Staphylococcyus aureus V8, α-chymotrypsine, "mouse sub-maxillary gland

protease" marketed by the BOEHRINGER company, Vibrio alginolyticus chemovar iophagus collagenase, which specifically recognises said peptides Gly-Pro and Gly-Ala, etc..

It is also possible to obtain polypeptides or fragments of envelope antigens of the virus, by cloning fragments excised from a cDNA constructed from genoimes of LAV variants.

Figures 2 and 3 are restriction maps of such a cDNA comprising a total of 9.1 to 9.2 kb. The polypeptides coded by cDNA fragments located in the region extending between site KpnI (position 6100) and site BgIII (position 9150) of the restriction map of Figure 2. The presence of a characteristic site of an envelope antigen of the LAV virus or the like in any polypeptide expressed (in a suitable host cell transformed behorehand by a corresponding fragment or by a vector containing said fragment) can be detected by any suitable immunochemical means.

Particularly the invention relates more particularly to polypeptides encoded by cDNA fragments defined hereafter. It also relates to the nucleic acid fragments themselves, including a cDNA variant corresponding to a whole LAV retroviral genome, characterized by a series of restriction sites in the order hereafter (from the 5' end to the 3' end).

The coordinates of the successive sites of the whole LAV genome (see also restriction map of λJ19 in fig. 1) are indicated hereafter too, with respect to the Hind III site (selected as of coordinate 1) which is located in the R region. The coordinates are estimated with an accuracy of ± 200 bp :

Hind III	0
Sac I	50
Hind III	520
Pst I	800

Hind III	1 100
Bgl II	1 500
Kpn I	3 500
Kpn I	3 900
Eco RI	4 100
Eco RI	5 300
Sal I	5 500
Kpn I	6 100
Bgl II	6 500
Bgl II	7 600
Hind III	7 850
Bam HI	8 150
Xho I	8 600
Kpn I	8 700
Bgl II	8 750
Bgl II	9 150
Sac I	9 200
Hind III	9 250

Another DNA variant according to this invention optionally contains an additional Hind III approximately at the 5 550 coordinate.

Reference is further made to fig. 1 which shows a more detailed restriction map of said whole-DNA (λJ19).

An even more detailed nucleotidic sequence of a preferred DNA according to the invention is shown in figs. 4a-4e hereafter.

The invention further relates to other preferred DNA fragments and polypeptide sequences (glycosylated or not glycosylated) which will be referred to hereafter.

SEQUENCING OF LAV

The sequencing and determination of sites of particular interest were carried out on a phage recombinant corresponding to λJ19 disclosed in the abovesaid British Patent application Nr. 84 23659. A method for preparing it is disclosed in that application.

The whole recombinant phage DNA of clone λJ19 (disclosed in the earlier application) was sonicated according to the protocol of DEININGER (1983), Analytical Biochem. 129, 216. the DNA was repaired by a Klenow reaction for 12 hours at 16°C. The DNA was electrophoresed through 0.8 % agarose gel and DNA in the size range of 300-600 bp was cut out and electroeluted and precipitated. Resuspended DNA (in 10 mM Tris, pH 8 ; 0,1 mM EDTA) was ligated into M13mp8 RF DNA (cut by the restriction enzyme SmaI and subsequently alkaline phosphated), using T4 DNA- and RNA-ligases (Maniatis T et al (1982) - Molecular cloning - Cold Spring Harbor Laboratory). An E. coli strain designated as TG1 was used for further study. This strain has the following genotype :

Δlac pro, supE, thi.F'traD36, proAB, $lacI^q$, ZΔM15,r^-

This E. coli TGI strain has the peculiarity of enabling recombinants to be recognized easily. The blue colour of the cells transfected with plasmids which did not recombine with a fragment of LAV DNA is not modified. To the contrary cells transfected by a recombinant plasmid containing a LAV DNA fragment yield white colonies. The technique which was used is disclosed in Gene (1983), 26, 101.

This strain was transformed with the ligation mix using the Hanahan method (Hanahan D (1983) J. Mol. Biol. 166, 557). Cells were plated out on tryptone-agarose plate with IPTG and X-gal in soft agarose. White plaques were either picked and screened or screened directly in situ using nitrocellulose filters. Their DNAs were hybridized with nick-translated DNA inserts of pUC18 Hind III subclones of λJ19. This permitted the isolation of the plasmids or subclones of λ which are identified in the table hereafter. In relation to this table it should also be noted that the designation of each plasmid is followed by the deposition number of a cell culture of E. coli TGI containing the corresponding plasmid at the "Collection

Nationale des Cultures de Micro-organismes" (C.N.C.M.) of the Pasteur Institute in Paris, France. A non-transformed TGI cell line was also deposited at the C.N.C.M. under Nr. I-364. All these deposits took place on November 15, 1984. The sizes of the corresponding inserts derived from the LAV genome have also been indicated.

TABLE

Essential features of the recombinant plasmids

- pJ19 - 1 plasmid (I-365) 0.5 kb

Hind III - Sac I - Hind III

- pJ19 - 17 plasmid (I-367) 0.6 kb

Hind III - Pst 1 - Hind III

- pJ19 - 6 plasmid (I-366) 1.5 kb

Hind III (5')
Bam HI
Xho I
Kpn I
Bgl II
Sac I (3')
Hind III

- pJ19-13 plasmid (I-368) 6.7 kb

Hind III (5')
Bgl II
Kpn I
Kpn I
Eco RI
Eco RI
Sal I
Kpn I
Bgl II
Bgl II
Hind III (3')

Positively hybridizing M13 phage plates were grown up for 5 hours and the single-stranded DNAs were extracted.

M13mp8 subclones of λJ19 DNAs were sequenced according to the dideoxy method and technology devised by Sanger et al (Sanger et al (1977), Proc. Natl. Acad. Sci. USA, 74 , 5463 and M13 cloning and sequencing handbook, AMERSHAM (1983). the 17-mer oligonucleotide primer α-^{35}SdATP (400Ci/mmol, AMERSHAM), and 0.5X-5X buffer gradient gels (Biggen M.D. et al (1983, Proc. Natl. Acad. Sci. USA, 50, 3963) were used. Gels were read and put into the computer under the programs of Staden (Staden R. (1982), Nucl. Acids Res. 10, 4731). All the appropriate references and methods can be found in the AMERSHAM M13 cloning and sequencing handbook.

The complete DNA sequence of λJ19 (also designated as LAV-Ia) is shown in figs. 4 to 4e.

The sequence was reconstructed from the sequence of phage λJ19 insert. The numbering starts at the cap site which was located experimentally (see hereafter). Important genetic elements, major open reading frames and their predicted products are indicated together with the HindIII cloning sites. The potential glycosylation sites in the env gene are overlined. The NH_2-terminal sequence of $p25^{gag}$ determined by protein microsequencing is boxed.

Each nucleotide was sequenced on average 5.3 times : 85 % of the sequence was determined on both strands and the remainder sequenced at least twice from independent clones. The base composition is T, 22.2 % ; C, 17.8 % ; A, 35.8 % ; G, 244.2 % ; G + C, 42 %. The dinucleotide GC is greatly under represented (0,9 %) as common amongst eukaryotic sequences (Bird 1980).

Figs. 5 and 6 provide a diagrammatized representation of the lengths of the successive open reading frames corresponding to the successive reading phases (also referred to by numbers "1", "2" and "3" appearing in

the left handside part of fig. 5). The relative positions of these open reading frames (ORF) with respect to the nucleotidic structure of the LAV genome is referred to by the scale of numbers representative of the respective positions of the corresponding nucleotides in the DNA sequence. The vertical bars correspond to the positions of the corresponding stop codons.

The following genes and DNA fragments can be distinguished on the different reading frames shown. Reference is then also made to the proteins or glycoproteins encoded by said genes and fragments.

1) The "gag gene" (or ORF-gag)

The "gag gene" codes for core proteins.

gag : near the 5' extremity of the gag orf is a "typical" initiation codon (Kozak 1984) (position 336) which is not only the first in the gag orf, but the first from the cap site. The precursor protein is 500-aminoacids long. Calculated MW = 55841 agrees with the 55 kd gag precursor polypeptide. The N-terminal aminoacid sequence of the major core protein p25 is encoded by the nucleotide sequence starting from position 732 (fig. 5a). This formally makes the link between the cloned LAV genome and the immunologically characterized LAV p25 protein. The protein encoded 5' of the p25 coding sequence is rather hydrophilic. Its calculated MW of 14866 is consistent with that of the gag protein p18. The 3' part of the gag region codes probably for the retroviral nucleic acid binding protein (NBP). Indeed, like in HTLV-1 (Seiki et al., 1983) and RSV (Schwartz et al., 1983), the motif Cys-X_2-Cys-X_{8-9}-Cys common to all NBP (Orozlan et al., 1984) is found duplicated (nucleotides 1509 and 1572 in LAV sequence). Consistent with its function the putative NBP is extremely basic (17 % Arg + Lys).

Particularly it appears that a genomic fragment (ORF-gag) thought to code for the core antigens including the p25, p18 and p13 proteins is located between

nucleotidic position 312 (starting with 5' CTA GCG GAG 3') and nucleotidic position 1835 (ending by CTCG TCA CAA 3'). The structure of the peptides or proteins encoded by parts of said ORF is deemed to be that corresponding to phase 2.

The methionine aminoacid "M" coded by the ATG at position 336-338 is the probable initiation methionine of the gag protein precursor. The end of ORF-gag and accordingly of gag protein appears to be located at position 1835.

The beginning of p25 protein, thought to start by a Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Gln-Met-Val-His-.... aminoacid sequence is thought to be coded for by the nucleotidic sequence CCTATA..., starting at position 732.

The invention is thus more particularly concerned with and relates to

- the DNA sequence, extending from nucleotide 336 up to about nucleotide 1650, deemed to encode a p55 protein which is considered a containing aminoacid sequences corresponding to those of the core proteins p18 and p25 of the LAV virus ;
- the DNA sequence, extending from nucleotide 732 up to about nucleotide 1300, deemed to encode the p25 protein ;
- the DNA sequence, extending from about nucleotide 1371to about nucleotide 1650, deemed to encode the p13 protein ;
- the DNA sequence, extending from nucleotide 336 up to about nucleotide 611, deemed to encode the p18 protein;

The invention also relates to the purified polypeptides which have the aminoacid structures encoded by the abovesaid fragments, particularly the p13, p18, p25, p55 proteins or polypeptides which have the structures corresponding to those resulting from the direct translations of the DNA sequences or fragments which have been defined more specifically hereabove, which peptidic sequences flow directly from fig. 4a. More particularly the invention relates to purified polypeptides having

peptidic sequences identical or equivalent to those encoded by the DNA sequences extending from the following nucleotide positions :

- 336 to 1650 (p55)
- 336 to 611 (p18)
- 1371 to 1650 (p13)
- 732 to 1300 (p25).

It should be mentioned that the p13, p18 and p25 all appear to derive from a same precursor, i.e. p55.

The invention further concerns polypeptide fragments encoded by corresponding DNA fragments of the gag open reading frame. Particularly hydrophilic peptides in the gag open reading frame are identified hereafter. They are defined starting from aminocid 1 = Met coded by the ATG starting from 336-338 in the LAV DNA sequence (fig. 3a) and then further numbered in accordance with their order in the gag sequence. The first and second numbers in relation to each peptide refer to the respective N-terminal and C-terminal-aminoacid respectively.

Those hydrophilic peptides include :

aminoacids 12-32 inclusive, i.e. Glu-Leu-Asp-Arg-Trp-Glu-Lys-Ile-Arg-Leu-Arg-Pro-Gly-Gly-Lys-Lys-Lys-Tyr-Lys-Leu-Lys

aminoacids 37-46 inclusive, i.e. Ala-Ser-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-

aminoacids 49-79 inclusive, i.e. Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr-Gly-Ser-Glu-Glu-Leu-Arg-Ser-Leu-Tyr-

aminoacids 88-153 inclusive, i.e. Val-His-Gln-Arg-Ile-Glu-Ile-Lys-Asp-Thr-Lys-Glu-Ala-Leu-Asp-Lys-Ile-Glu-Glu-Glu-Gln-Asn-Lys-Ser-Lys-Lys-Lys-Ala-Gln-Gln-Ala-Ala-Ala-Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Gln-Met-Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-

aminocacids 158-165 inclusive, i.e. Val-Val-Glu-Glu-Lys-Ala-Phe-Ser-

aminoacids 178-188 inclusive, i.e. Gly-Ala-Thr-Pro-Gln-Asp-Leu-Asn-Thr-Met-Leu-

aminoacids 200-220 inclusive, i.e. Met-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-

aminoacids 226-234 inclusive, i.e. Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-

aminoacids 239-264 inclusive, i.e. Thr-Thr-Ser-Thr-Leu-Gln-Glu-Gln-Ile-Gly-Trp-Met-Thr-Asn-Asn-Pro-Pro-Ile-Pro-Val-Gly-Glu-Ile-Tyr-Lys-Arg-

aminocids 288-331 inclusive, i.e. Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Ala-Glu-Gln-Ala-Ser-Gln-Glu-Val-Lys-Asn-Trp-Met-Thr-GluThr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-

aminoacids 352-361 inclusive, i.e. Gly-Val-Gly-Gly-Pro-Gly-His-Lys-Ala-Arg-

aminoacids 377-390 inclusive, i.e. Met-Met-Gln-Arg-Gly-Asn-Phe-Arg-Asn-Gln-Arg-Lys-Ile-Val-

aminoacids 399-432 inclusive, i.e. Gly-His-Ile-Ala-Arg-Asn-Cys-Arg-Ala-Pro-Arg-Lys-Lys-Gly-Cys-Trp-Lys-Cys-Gly-Lys-Glu-Gly-His-Gln-Met-Lys-Asp-Cys-Thr-Glu-Arg-Gln-Ala-Asn-

aminoacids 437-484 inclusive, i.e. Ile-Trp-Pro-Ser-Tyr-Lys-Gly-Arg-Pro-Gly-Asn-Phe-Leu-Gln-Ser-Arg-Pro-Glu-Pro-Thr-Ala-Pro-Pro-Glu-Glu-Ser-Phe-Arg-Ser-Gly-Val-Glu-Thr-Thr-Thr-Pro-Ser-Gln-Lys-Gln-Glu-Pro-Ile-Asp-Lys-Glu-Leu-Tyr-

aminoacids 492-498 inclusive, i.e. Leu-Phe-Gly-Asn-Asp-Pro-Ser-

The invention also relates to any combination of these peptides.

2) The "pol gene" (or ORF-pol)

Pol : The reverse transcriptase gene can encode a protein of up to 1,003 aminoacids (calculated MW = 113629). Since the first methionine codon is 92 triplets from the origin of the open reading frame, it is possible that the protein is translated from a spliced messenger RNA, so giving a gag-pol polyprotein precursor.

The pol coding region is the only one in which significant homology has been found with other retroviral protein sequences, three domains of homology being apparent. The first is a very short region of 17 amino-acids (starting at 1856). Homologous regions are located within the p15 gag^{RSV} protease (Dittmar and Moelling 1978) and a polypeptid encoded by an open reading frame located between gag and pol of HTLV-1 (fig. 5) (Schwartz et al., 1983, Seiki et al., 1983). This first domain could thus correspond to a conserved sequence in viral proteases. Its different location within the three genomes may not be significant since retroviruses, by splicing or other mechanisms, express a gag-pol polyprotein precursor (Schwartz et al., 1983, Seiki et al., 1983). The second and most extensive region of homology (starting at 2048) probably represents the core sequence of the reverse transcriptase. Over a region of 250 aminoacids, with only minimal insertions or deletions, LAV shows 38 % aminoacid identity with RSV, 25 % with HTLV-I, 21 % with MoMuLV (Schinnick et al., 1981) while HTLV-I and RSV show 38 % identity in the same region. A third homologous region is situated at the 3' end of the pol reading frame and corresponds to part of the pp32 peptide of RSV that has exonuclease activity (Misra et al., 1982). Once again, there is greater homology with the corresponding RSV sequence than with HTLV-1.

Figs. 4a-4c also show that the DNA fragment extending from nucleotidic position 1631 (starting with 5'TTT TTT ....3' to nucleotidic position 5162 thought to

correspond to the pol gene. The polypeptidic structure of the corresponding polypeptides is deemed to be that corresponding to phase 1. It stops at position 4639 (end by 5'G GAT GAG GAT 3').

These genes are thought to code for the virus polymerase or reverse transcriptase.

3) The envelope gene (or ORF-env)

env : The env open reading frame has a possible initiator methionine codon very near the beginning (8th triplet). If so the molecular weight of the presumed env precursor protein (861 aminoacids, MWcalc = 97376) is consistent with the size of the LAV glycoprotein (110 kd and 90 kd after glycosidase treatment). There are 32 potential N-glycosylation sites (Asn-X-Ser/Thr) which are overlined in Fig. 4d and 4e. An interesting feature of env is the very high number of Trp residues at both ends of the protein.

The DNA sequence thought to code for envelope proteins is thought to extend from nucleotidic position 5746 (starting with 5' AAA GAG GAG A....3') up to nucleotidic position 8908 (ending by .....A ACT AAA GAA 3'). Polypeptidic structures of sequences of the envelope protein correspond to those read according to the "phase 3" reading phase.

The start of env transcription is thought to be at the level of the ATG codon at position 5767-5769.

There are three hydrophobic regions, characteristic of the retroviral envelope proteins (Seiki et al., 1983) corresponding to a signal peptide (encoded by nucleotides 5815-5850 bp), a second region (7315-7350 bp) and a transmembrane segment (7831-7890 bp). The second hydrophobic region (7315-7350 bp) is preceeded by a stretch rich in Arg + Lys. It is possible that this represents a site of proteolytic cleavage, which by analogy with over retroviral proteins, would give an external envelope polypeptide and a membrane associated protein

(Seiki et al., 1983, Kiyokawa et al., 1984). A striking feature of the LAV envelope protein sequence is that the segment encoding the transmembrane protein is of unusual length (150 residues). The env protein shows no homology to any sequence in protein data banks. The small aminoacid motif common to the transmembrane proteins of all leukemogenic retroviruses (Cianciolo et al., 1984) is not present in lav env.

The invention concerns more particularly the DNA sequence extending from nucleotide 5767 up to nucleotide 7314 deemed to encode the gp 110 (envelope glycoprotein of the LAV virus which has a molecular weight of about 110,000 daltons) beginning at about nucleotide as well as the polypeptidic backbone of the glycoprotein sequence which corresponds to that having an approximate molecular weight which was initially believed to be 90,000 daltons, and which turned out to be 55,000. The polypeptide resulting from the complete removal of sugar residues of gp110 can be obtained by the treatment of said gp110 with the appropriate glycosidase.

The invention further relates to the purified polypeptides which have the aminoacid structure (or polypeptidic backbone) of the gp110 and gp90, which correspond to the direct translation of the DNA sequences and fragments which have been defined more specifically hereabove (figs 4d and 4e).

The invention further relates to polypeptides containing neutralizing epitopes.

The locations of neutralizing epitopes are further apparent in fig. 4d. Reference is more particularly made to the overlined groups of three letters included in the aminoacid sequences of the envelope proteins (reading phase 3) which can be designated generally by the formula Asn-X-Ser or Asn-X-Thr, wherein X is any other possible aminoacid. Thus the initial protein product or polypeptide backbone of the env glycoprotein has a molecular weight in excess of 91,000. These groups are deemed to generally carry glycosylated groups. These Asn-X-Ser and Asn-X-Thr groups with attached glycosylated groups form together hydrophylic regions of the protein and are deemed to be

located at the periphery of and to be exposed outwardly with respect to the normal conformation of the proteins. Consequently they are considered as being epitopes which can efficiently be brought into play in vaccine compositions.

The invention thus concerns with more particularity peptide sequences included in the env-proteins and excizable therefrom (or having the same aminoacid structure), having sizes not exceeding 200 aminoacids.

Preferred peptides of this invention (referred to hereafter as a, b, c, d, e, f) are deemed to correspond to those encoded by the nucleotide sequences which extend respectively between the following positions :

a) from about 6171 to about 6276
b) " " 6336 " " 6386
c) " " 6466 " " 6516
d) " " 6561 " " 6696
e) " " 6936 " " 7006
f) " " 7611 " " 7746

Other hydrophilic peptides in the env open reading frame are identified hereafter. they are defined starting from aminoacid 1 = lysine coded by the AAA at position 5746-5748 in the LAV DNA sequence (figs 4d and 4e) and then further numbered in accordance with their order with respect to the end sequence. The first and second numbers in relation to each peptide refer to their respective N-terminal and C-terminal aminoacids.

These hydrophilic peptides are :

aminoacids 8-23 inclusive, i.e. Met-Arg-Val-Lys-Glu-Lys-Tyr-Gln-His-Leu-Trp-Arg-Trp-Gly-Trp-Lys-

aminoacids 63-78 inclusive, i.e. Ser-Asp-Ala-Lys-Ala-Tyr-Asp-Thr-Glu-Val-His-Asn-Val-Trp-Ala-Thr-

aminoacids 82-90 inclusive, i.e. Val-Pro-Thr-Asp-Pro-Asn-Pro-Gln-Glu-

aminoacids 97-123 inclusive, i.e. Thr-Glu-Asn-Phe-Asn-Met-Trp-Lys-Asn-Asp-Met-Val-Glu-Gln-Met-His-Glu-Asp-Ile-Ile-Ser-Leu-Trp-Asp-Gln-Ser-Leu-

aminocids 127-183 inclusive, i.e. Val-Lys-Leu-Thr-Pro-Leu-Cys-Val-Ser-Leu-Lys-Cys-Thr-Asp-Leu-Gly-Asn-Ala-Thr-Asn-Thr-Asn-Ser-Ser-Asn-Thr-Asn-Ser-Ser-Ser-Gly-Glu-Met-Met-Met-Glu-Lys-Gly-Glu-Ile-Lys-Asn-Cys-Ser-Phe-Asn-Ile-Ser-Thr-Ser-Ile-Arg-Gly-Lys-Val-Gln-Lys-

aminoacids 197-201 inclusive, i.e. Leu-Asp-Ile-Ile-Pro-Ile-Asp-Asn-Asp-Thr-Thr-

aminocids 239-294 inclusive, i.e. Lys-Cys-Asn-Asn-Lys-Thr-Phe-Asn-Gly-Thr-Gly-Pro-Cys-Thr-Asn-Val-Ser-Thr-Val-Gln-Cys-Thr-His-Gly-Ile-Arg-Pro-Val-Val-Ser-Thr-Gln-Leu-Leu-Leu-Asn-Gly-Ser-Leu-Ala-Glu-Glu-Glu-Val-Val-Ile-Arg-Ser-Ala-Asn-Phe-Thr-Asp-Asn-Ala-Lys-

aminocids 300-327 inclusive,i.e. Leu-Asn-Gln-Ser-Val-Glu-Ile-Asn-Cys-Thr-Arg-Pro-Asn-Asn-Asn-Thr-Arg-Lys-Ser-Ile-Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-

aminoacids 334-381 inclusive, i.e. Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg-Ala-Lys-Trp-Asn-Ala-Thr-Leu-Lys-Gln-Ile-Ala-Ser-Lys-Leu-Arg-Glu-Gln-Phe-Gly-Asn-Asn-Lys-Thr-Ile-Ile-Phe-Lys-Gln-Ser-Ser-Gly-Gly-Asp-Pro-

aminoacids 397-424 inclusive, i.e. Cys-Asn-Ser-Thr-Gln-Leu-Phe-Asn-Ser-Thr-Trp-Phe-Asn-Ser-Thr-Trp-Ser-Thr-Glu-Gly-Ser-Asn-Asn-Thr-Glu-Gly-Ser-Asp-

aminoacids 466-500 inclusive, i.e. Leu-Thr-Arg-Asp-Gly-Gly-Asn-Asn-Asn-Asn-Gly-Ser-Glu-Ile-Phe-Arg-Pro-Gly-Gly-Gly-Asp-Met-Arg-Asp-Asn-Trp-Arg-Ser-Glu-Leu-Tyr-Lys-Tyr-Lys-Val-

aminoacids 510-523 inclusive, i.e. Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-

aminoacids 551-577 inclusive, i.e. Val-Gln-Ala-Arg-Gln-Leu-Leu-Ser-Gly-Ile-Val-Gln-Gln-Gln-Asn-Asn-Leu-Leu-Arg-Ala-Ile-Glu-Ala-Gln-Gln-His-Leu-

aminoacids 594-603 inclusive, i.e. Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-

aminoacids 621-630 inclusive, i.e. Pro-Trp-Asn-Ala-Ser-Trp-Ser-Asn-Lys-Ser-

aminoacids 657-679 inclusive, i.e. Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-

aminoacids 719-758 inclusive, i.e. Arg-Val-Arg-Gln-Gly-Tyr-Ser-Pro-Leu-Ser-Phe-Gln-Thr-His-Leu-Pro-Thr-Pro-Arg-Gly-Pro-Asp-Arg-Pro-Glu-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Glu-Arg-Asp-Arg-Asp-Arg-Ser-Ile-

aminoacids 780-803 inclusive, i.e. Tyr-His-Arg-Leu-Arg-Asp-Leu-Leu-Leu-Ile-Val-Thr-Arg-Ile-Val-Glu-Leu-Leu-Gly-Arg-Arg-Gly-Trp-Glu-

The invention also relates to any combination of these peptides.

4) <u>The other ORFs</u>

The invention further concerns DNA sequences which provide open reading frames defined as ORF-Q, ORF-R and as "1", "2", "3", "4", "5", the relative positions of which appear more particularly in figs. 2 and 3.

These ORFs have the following locations :

ORF-Q	phase	1	start	4554	stop	5162
ORF-R	"	2	"	8325	"	8972
ORF-1	"	1	"	5105	"	5392
ORF-2	"	2	"	5349	"	5591
ORF-3	"	1	"	5459	"	5692
ORF-4	"	2	"	5595	"	5849
ORF-5	"	1	"	8042	"	8355

<u>ORFs Q and F</u>

The viral (+) strand of the LAV genome was found to contain the statutory retroviral genes encoding the core structural proteins (gag), reverse transcriptase

(pol) and envelope protein (env), and two extra open reading frames (orf) which we call Q and F (Table 1). The genetic organization of LAV, 5'LTR-gag-pol-Q-env-F-3'LTR, is unique. Whereas in all replication competent retroviruses pol and env genes overlap, in LAV they are separated by orf Q (192 amino acids) followed by four small (<100 triplets) orf. The orf F (206 amino acids) slightly overlaps the 3' end of env and is remarkable in that it is half encoded by the U3 region of the LTR.

Such a structure places LAV clearly apart from previously sequenced retroviruses (Fig. 2). the (-) strand is apparently non coding. The additional HindIII site of the LAV clone λJ81 (with respect to λJ19) maps to the apparently non-coding region between Q and env (positions 5166-5745). Starting at position 5501 is a sequence (AAGCCT) which differs by a single base (underlined) from the HindIII recognition sequence. It is to be anticipated that many of the restriction site polymorphism between different isolates will map to this region. Clone λJ81 has also been referred to in British application Nr. 84 23659 filed on September 15, 1984.

Q and F :

The nucleotide positions of their respective extremities are given in Table 1 hereafter.

The location of orf Q is without precedent in the structure of retroviruses. Orf F is unique in that it is half encoded by the U3 element of the LTR. Both orfs have "strong" initiator codons (Kozak 1984) near their 5' ends and can encode proteins of 192 aminoacids (MW calc = 22487) and 206 aminoacids (MWcalc = 23316) respectively. Both putative proteins are hydrophilic (pQ 49 % polar, 15.1 % Arg + Lys : pF 46 % polar, 11 % Arg + Lys) and are therefore unlikely to be associated directly with membrane. The function for the putative proteins pQ and pF cannot be predicted as no homology was found by screening protein sequence data banks. Between orf F and the pX

protein of HTLV-1 there is no detectable homology. Furthermore their hydrophobicity/hydrophilicity profiles are completely different. It is known that retroviruses can transduce cellular genes notably proto-oncogenes (Weinberg 1982)). We suggest that orfs Q and F represent exogenous genetic material and not some vestige of cellular DNA because (I) LAV DNA does not hybridize to the human genome under stringent conditions (Alizon et al., 1984), (II) their codon usage is comparable to that of the gag, pol and env genes (data not shown).

The organization of a reconstructed LTR and viral flanking elements are shown schematically in Fig. 6. The LTR is 638 bp long and displays usual features (Chen and Barker 1984) : (I) It is bounded by an inverted repeat (5'ACTG) including the conserved TG dinucleotide (Temin 1981), (II) Adjacent to 5' LTR is the tRNA primer binding site (PBS), complementary to $tRNA_3^{lys}$ (Raba et al., 1979), (III) adjacent to 3'LTR is a perfect 15 bp polypurine tract. The other three polypurine tracts observed between nucleotides 8200-8800 are not followed by a sequence which is complementary to that just preceeding the PBS. The limits of U5, R and U3 elements were determined as follows. U5 is located between PBS and the polyadenylation site established from the sequence of the 3' end of oligo(dT)-primed LAVcDNA (Alizon et al., 1984). Thus U5 is 84 bps long. The length of R+U5 was determined by synthesizing tRNA-primed LAV cDNA. After alkaline hydrolysis of the primer, R+U5 was found to be 181 ± 1 bp. Thus R is 97 bps long and the capping site at its 5' end can be located. Finally U3 is 456 bp long. The LAV LTR also contains characteristic regulatory elements : a polyadenylation signal sequence AATAAA 19 bp from the R-U5 junction and the sequence ATATAAG which is very likely the TATA box, 22 bps 5' of the cap site.There are no longer direct repeats

within the LTR. Interestingly the LAV LTR shows some similarities to that of the mouse mammary tumour virus (MMTV) Donehower et al., 1981). They both use $tRNA_3^{lys}$ as a primer for (-) strand synthesis whereas all other exogenous mammalian retroviruses known to date use $tRNA^{pro}$ (Chen and Barker 1984). They possess very similar polypurine tracts (that of LAV is AAAAGAAAAGGGGGG while that of MMTV is AAAAAAGAAAAAGGGGG). It is probable that the viral (+) strand synthesis is discontinuous since the polypurine tract flanking the U3 element of the 3' LTR is found exactly duplicated in the 3' end of orf pol, at 4331-4336. In addition, MMTV and LAV are exceptionnal in that the U3element can encode an orf. In the case of MMTV, U3 contains the whole orf while in LAV, U3 contains 110 codons of the 3' half of orf F.

The LAV long terminal repeat (LTR) is diagrammatically represented in Fig. 6. As mentioned the LTR was reconstructed from the sequence of λJ19 by juxtaposing the sequences adjacent to the HindIII cloning sites.

Sequencing of oligo(dT) primed LAV DNA clone pLAV75 (Alizon et al., 1984) rules out the possibility of clustered HindIII sites in the R region of LAV. LTR are limited by an invertd repeat sequence (IR). Both of the viral elements flanking the LTR have been represented = tRNA primer binding site (PBS) for 5' LTR and polypurine track (PU) for 3' LTR. Also indicated are a putative TATA box, the cap site, polydenylation signal (AATAAA) and polyadenylation site (CAA). The location of the open reading frame F (648 nucleotides) is shown above the LTR schema.

The LTR (long terminal repeats) can also be defined as lying between position 8560 and position 160 (end extending over position 9097/1). As a matter of fact the end of the genome is at 9097 and, because of the LTR structure of the retrovirus, links up with the beginning

of the sequence :

Hind III

CTCAATAAAGCTTGCCTTG

9097 1

Table 1 sums up the locations and sizes of viral open reading frames. The nucleotide coordinates refer to the first base of the first triplet (1st triplet), of the first methionine initiation codon (Met) and of the stop codon (stop). The number of aminoacids and calculated molecular weights are those calculated for unmodified precursor products starting at the first methionine through to the end with the exception of pol where the size and MW refer to that of the whole orf.

orf	1st triplet	Met	stop	No amino acids	MWcalc
gag	312	336	1836	500	55841
pol	1631	1934	4640	(1003)	(113629)
orf Q	4554	4587	5163	192	22487
env	5746	5767	8350	861	97376
orf F	8324	8354	8972	206	23316

Table 1 : Location and sizes of viral open reading frames.

The invention concerns more particularly all the DNA fragments which have been more specifically referred to hereabove and which correspond to open reading frames. It will be understood that the man skilled in the art will be able to obtain them all, for instance by cleaving an entire DNA corresponding to the complete genome of a LAV species, such as by cleavage by a partial or complete digestion thereof with a suitable restriction enzyme and by the subsequent recovery of the relevant fragments. The different DNAs disclosed above can be resorted to also as a source of suitable fragments. The techniques disclosed hereafter for the isolation of the fragments which were then included in the plasmids referred to hereabove and which were then used for the DNA sequencing can be used.

Of course other methods can be used. Some of them have been examplified in British Application Nr. 8423659 filed on September 19, 1984. Reference is for instance made to the following methods.

a) DNA can be transfected into mammalian cells with appropriate selection markers by a variety of techniques, calcium phosphate precipitation, polyethylene glycol, protoplast-fusion, etc..

b) DNA fragments corresponding to genes can be cloned into expression vectors for E. coli , yeast- or mammalian cells and the resultant proteins purified.

c) The provival DNA can be "shot-gunned" (fragmented) into procaryotic expression vectors to generate fusion polypeptides. Recombinant producing antigenically competent fusion proteins can be identified by simply screening the recombinants with antibodies against LAV antigens .

The invention further refers more specifically to DNA recombinants, particularly modified vectors, including any of the preceding DNA sequences and adapted to transform corresponding microorganisms or

cells, particularly eucaryotic cells such as yeasts, for instance saccharomyces cerevisiae, or higher eucaryotic cells, particularly cells of mammals, and to permit expression of said DNA sequences in the corresponding microorganisms or cells. General methods of that type have been recalled in the abovesaid British patent application Nr. 8429099 filed on November 16, 1984.

More particularly the invention relates to such modified DNA recombinant vectors modified by the abovesaid DNA sequences and which are capable of transforming higher eucaryotic cells particularly mammalian cells. Preferably any of the abovesaid sequences are placed under the direct control of a promoter contained in said vectors and which is recognized by the polymerases of said cells, such that the first nucleotide codons expressed correspond to the first triplets of the above-defined DNA-sequences. Accordingly this invention also relates to the corresponding DNA fragments which can be obtained from LAV genomas or corresponding cDNAs by any appropriate method. For instance such a method comprises cleaving said LAV genomas or cDNAs by restriction enzymes preferably at the level of restriction sites surrounding said fragments and close to the opposite extremities respectively thereof, recovering and identifying the fragments sought according to sizes, if need be checking their restriction maps or nucleotide sequences (or by reaction with monoclonal antibodies specifically directed against epitopes carried by the polypeptides encoded by said DNA fragments), and further if need be, trimming the extremities of the fragment, for instance by an exonucleolytic enzyme such as Bal31, for the purpose of controlling the desired nucleotide-sequences of the extremities of said DNA fragments or, conversely, repairing said extremities with Klenow enzyme and possibly ligating the latter to synthetic polynucleotide fragments designed to permit the reconstitution of the nucleotide

extremities of said fragments. Those fragments may then be inserted in any of said vectors for causing the expression of the corresponding polypeptide by the cell transformed therewith. The corresponding polypeptide can then be recovered from the transformed cells, if need be after lysis thereof, and purified, by methods such as electrophoresis. Needless to say that all conventionnal methods for performing these operations can be resorted to.

The invention also relates more specifically to cloned probes which can be made starting from any DNA fragment according to this invention, thus to recombinant DNAs containing such fragments, particularly any plasmids amplifiable in procaryotic or eucaryotic cells and carrying said fragments.

Using the cloned DNA fragments as a molecular hybridization probe - either by labelling with radionucleotides or with fluorescent reagents - LAV virion RNA may be detected directly in the blood, body fluids and blood products (e.g. of the antihemophylic factors such as Factor VIII concentrates) and vaccines, i.e. hepatitis B vaccine. It has already been shown that whole virus can be detected in culture supernatants of LAV producing cells. A suitable method for achieving that detection comprises immobilizing virus onto a support, e.g. nitrocellulose filters, etc., disrupting the virion and hybridizing with labelled (radiolabelled or "cold" - fluorescent- or enzyme-labelled) probes. Such an approach has already been developed for Hepatitis B virus in peripheral blood (according to SCOTTO J. et al. Hepatology (1983), 3, 379-384).

Probes according to the invention can also be used for rapid screening of genomic DNA derived from the tissue of patients with LAV related symptoms, to see if the proviral DNA or RNA is present in host tissue and other tissues.

A method which can be used for such screening

comprises the following steps : extraction of DNA from tissue, restriction enzyme cleavage of said DNA, electrophoresis of the fragments and Southern blotting of genomic DNA from tissues, subsequent hybridization with labelled cloned LAV provival DNA. Hybridization in situ can also be used.

Lymphatic fluids and tissues and other non-lymphatic tissues of humans, primates and other mammalian species can also be screened to see if other evolutionnary related retrovirus exist. The methods referred to hereabove can be used, although hybridization and washings would be done under non stringent conditions.

The DNAs or DNA fragments according to the invention can be used also for achieving the expression of LAV viral antigens for diagnostic purposes.

The invention relates generally to the polypeptides themselves, whether synthetized chemically isolated from viral preparation or expressed by the different DNAs of the inventions, particularly by the ORFs or fragments thereof, in appropriate hosts, particularly procaryotic or eucaryotic hosts, after transformation thereof with a suitable vector previously modified by the corresonding DNAs.

More generally, the invention also relates to any of the polypeptide fragments (or molecules, particularly glycoproteins having the same polypeptidic backbone as the polypeptides mentioned hereabove) bearing an epitope characteristic of a LAV protein or glycoprotein, which polypeptide or molecule then has N-terminal and C-terminal extremities respectively either free or, independently from each other, covalently bond to aminoacids other than those which are normally associated with them in the larger polypeptides or glycoproteins of the LAV virus, which last mentioned aminoacids are then free or belong to another polypeptidic sequence. Particularly the invention relates to hybrid polypeptides containing any of

the epitope-bearing-polypeptides which have been defined more specifically hereabove, recombined with other polypeptides fragments normally foreign to the LAV proteins, having sizes sufficient to provide for an increased immunogenicity of the epitope-bearing-polypeptide yet, said foreign polypeptide fragments either being immunogenically inert or not interfering with the immunogenic properties of the epitope-bearing-polypeptide.

Such hybrid polypeptides which may contain up to 150, even 250 aminoacids usually consist of the expression products of a vector which contained ab initio a nucleic acid sequence expressible under the control of a suitable promoter or replicon in a suitable host, which nucleic acid sequence had however beforehand been modified by insertion therein of a DNA sequence encoding said epitope-bearing-polypeptide.

Said epitope-bearing-polypeptides, particularly those whose N-terminal and C-terminal aminoacids are free, are also accessible by chemical synthesis, according to technics well known in the chemistry of proteins.

The synthesis of peptides in homogeneous solution and in solid phase is well known.

In this respect, recourse may be had to the method of synthesis in homogeneous solution described by Houbenweyl in the work entitled "Methoden der Organischen Chemie" (Methods of Organic Chemistry) edited by E. WUNSCH., vol. 15-I and II, THIEME, Stuttgart 1974.

This method of synthesis consists of successively condensing either the successive aminoacids in twos, in the appropriate order or successive peptide fragments previously available or formed and containing already several aminoacyl residues in the appropriate order respectively. Except for the carboxyl and aminogroups which will be engaged in the formation of the peptide bonds, care must be taken to protect beforehand all other reactive groups borne by these aminoacyl groups

or fragments. However, prior to the formation of the peptide bonds, the carboxyl groups are advantageously activated, according to methods well known in the synthesis of peptides. Alternatively, recourse may be had to coupling reactions bringing into play conventional coupling reagents, for instance of the carbodiimide type, such as 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide. When the aminoacid group used carries an additional amine group (e.g. lysine) or another acid function (e.g. glutamic acid), these groups may be protected by carbobenzoxy or t-butyloxycarbonyl groups, as regards the amine groups, or by t-butylester groups, as regards the carboxylic groups. Similar procedures are available for the protection of other reactive groups. For example, SH group (e.g. in cysteine) can be protected by an acetamidomethyl or paramethoxybenzyl group.

In the case of progressive synthesis, aminoacid by aminoacid, the synthesis starts preferably by the condensation of the C-terminal aminoacid with the aminoacid which corresponds to the neighboring aminoacyl group in the desired sequence and so on, step by step, up to the N-terminal aminoacid. Another preferred technique can be relied upon is that described by R.D. Merrifield in "solid phase peptide synthesis" (J. Am. Chem. Soc., 45, 2149-2154).

In accordance with the Merrifield process, the first C-terminal aminoacid of the chain is fixed to a suitable porous polymeric resin, by means of its carboxylic group, the amino group of said aminoacid then being protected, for example by a t-butyloxycarbonyl group.

When the first C-terminal aminoacid is thus fixed to the resin, the protective group of the amine group is removed by washing the resin with an acid, i.e. trifluoroacetic acid, when the protective group of the amine group is a t-butyloxycarbonyl group.

Then the carboxylic group of the second

aminoacid which is to provide the second aminoacyl group of the desired peptidic sequence, is coupled to the deprotected amine group of the C-terminal aminoacid fixed to the resin. Preferably, the carboxyl group of this second aminoacid has been activated, for example by dicyclohexylcarbodiimide, while its amine group has been protected, for example by a t-butyloxycarbonyl group. The first part of the desired peptide chain, which comprising the first two aminoacids, is thus obtained. As previously, the amine group is then deprotected, and one can further proceed with the fixing of the next aminoacyl group and so forth until the whole peptide sought is obtained.

The protective groups of the different side groups, if any, of the peptide chain so formed can then be removed. The peptide sought can then be detached from the resin, for example, by means of hydrofluoric acid, and finally recovered in pure form from the acid solution according to conventional procedures.

As regards the peptide sequences of smallest size and bearing an epitope or imunogenic determinant, and more particularly those which are readily accessible by chemical synthesis, it may be required, in order to increase their in vivo immunogenic character, to couple or "conjugate" them covalently to a physiologically acceptable and non toxic carrier molecule.

By way of examples of carrier molecules or macromolecular supports which can be used for making the conjugates according to the invention, will be mentioned natural proteins, such as tetanic toxoid, ovalbumin, serum-albumins, hemocyanins, etc.. Synthetic macromolecular carriers, for example polysines or poly(D-L-alanine)-poly(L-lysine)s, can be used too.

Other types of macromolecular carriers which can be used, which generally have molecular weights higher than 20,000, are known from the literature.

The conjugates can be synthesized by known

processes, such as described by Frantz and Robertson in "Infect. and Immunity", 33, 193-198 (1981), or by P.E. Kauffman in Applied and Environmental Microbiology, October 1981, Vol. 42, n° 4, 611-614.

For instance the following coupling agents can be used : glutaric aldehyde, ethyl chloroformate, water-soluble carbodiimides (N-ethyl-N'(3-dimethylamino-propyl) carbodiimide, HCl), diisocyanates, bis-diazobenzidine, di- and trichloro-s-triazines, cyanogen bromides, benzaquinone, as well as coupling agents mentioned in "Scand. J. Immunol., 1978, vol. 8, p. 7-23 (Avrameas, Ternynck, Guesdon).

Any coupling process can be used for bonding one or several reactive groups of the peptide, on the one hand, and one or several reactive groups of the carrier, on the other hand. Again coupling is advantageously achieved between carboxyl and amine groups carried by the peptide and the carrier or vice-versa in the presence of a coupling agent of the type used in protein synthesis, i.e. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, N-hydroxybenzotriazole, etc.. Coupling between amine groups respectively borne by the peptide and the carrier can also be made with glutaraldehyde, for instance, according to the method described by BOQUET, P. et al. (1982) Molec. Immunol., 19, 1441-1549, when the carrier is hemocyanin.

The immunogenicity of epitope-bearing-peptides can also be reinforced, by oligomerisation thereof, for example in the presence of glutaraldehyde or any other suitable coupling agent. In particular, the invention relates to the water soluble immunogenic oligomers thus obtained, comprising particularly from 2 to 10 monomer units.

The glycoproteins, proteins and polypeptides (generally designated hereafter as "antigens" of this invention, whether obtained in a purified state from LAV virus preparations or - as concerns more particularly the

peptides - by chemical synthesis, are useful in processes for the detection of the presence of anti-LAV antibodies in biological media, particularly biological fluids such as sera from man or animal, particularly with a view of possibly diagnosing LAS or AIDS.

Particularly the invention relates to an in vitro process of diagnosis making use of an envelope glycoprotein (or of a polypeptide bearing an epitope of this glycoprotein) for the detection of anti-LAV antibodies in the serums of persons who carry them. Other polypeptides - particular those carrying an epitope of a core protein - can be used too.

A preferred embodiment of the process of the invention comprises :

- depositing a predetermined amount of one or several of said antigens in the cups of a titration miroplate ;
- introducing of increasing dilutions of the biological fluid, i.e. serum to be diagnosed into these cups :
- incubating the microplate :
- washing carefully the microplate with an appropriate buffer :
- adding into the cups specific labelled antibodies directed against blood immunoglobulins and
- detecting the antigen-antibody-complex formed, which is then indicative of the presence of LAV antibodies in the biological fluid.

Advantageously the labelling of the anti-immunoglobulin antibodies is achieved by an enzyme selected from among those which are capable of hydrolysing a substrate, which substrate undergoes a modification of its radiation-absorption, at least within a predetermined band of wavelenghts. The detection of the substrate, preferably comparatively with respect to a control, then provides a measurement of the potential risks or of the effective presence of the disease.

Thus preferred methods immuno-enzymatic or

also immunofluorescent detections, in particular according to the ELISA technique. Titrations may be determinations by immunofluorescence or direct or indirect immunoenzymatic determinations. Quantitative titrations of antibodies on the serums studied can be made.

The invention also relates to the diagnostic kits themselves for the in vitro detection of antibodies against the LAV virus, which kits comprise any of the polypeptides identified herein, and all the biological and chemical reagents, as well as equipment, necessary for peforming diagnostic assays. Preferred kits comprise all reagents required for carrying out ELISA assays. Thus preferred kits will include, in addition to any of said polypeptides, suitable buffers and anti-human immunoglobulins, which anti-human immunoglobulins are labelled either by an immunofluorescent molecule or by an enzyme. In the last instance preferred kits then also comprise a substrate hydrolysable by the enzyme and providing a signal, particularly modified absorption of a radiation, at least in a determined wavelength, which signal is then indicative of the presence of antibody in the biological fluid to be assayed with said kit.

The invention also relates to vaccine compositions whose active principle is to be constituted by any of the antigens, i.e. the hereabove disclosed polypeptides whole antigens, particularly the purified gp110 or immunogenic fragments thereof, fusion polypeptides or oligopeptides in association with a suitable pharmaceutical or physiologically acceptable carrier.

A first type of preferred active principle is the gp110 immunogen.

Other preferred active principles to be considered in that fields consist of the peptides containing less than 250 aminoacid units, preferably less than 150, as deducible for the complete genomas of LAV, and even more preferably those peptides which contain one or more

groups selected from Asn-X-Ser and Asn-X-Ser as defined above. Preferred peptides for use in the production of vaccinating principles are peptides (a) to (f) as defined above. By way of example having no limitative character, there may be mentioned that suitable dosages of the vaccine compositions are those which are effective to elicit antibodies in vivo, in the host, particularly a human host. Suitable doses range from 10 to 500 micrograms of polypeptide, protein or glycoprotein per kg, for instance 50 to 100 micrograms per kg.

The different peptides according to this invention can also be used themselves for the production of antibodies, preferably monoclonal antibodies specific of the different peptides respectively. For the production of hybridomas secreting said monoclonal antibodies, conventional production and screening methods are used. These monoclonal antibodies, which themselves are part of the invention then provide very useful tools for the identification and even determination of relative proportions of the different polypeptides or proteins in biological samples, particularly human samples containing LAV or related viruses.

The invention further relates to the hosts (procaryotic or eucaryotic cells) which are transformed by the above mentioned recombinants and which are capable of expressing said DNA fragments.

Finally the invention also concerns vectors for the transformation of eucaryotic cells of human origin, particularly lymphocytes, the polymerases of which are capable of recognizing the LTRs of LAV. Particularly said vectors are characterized by the presence of a LAV LTR therein, said LTR being then active as a promoter enabling the efficient transcription and translation in a suitable host of a DNA insert coding for a determined protein placed under its controls.

Needless to say that the invention extends to

all variants of genomes and corresponding DNA fragments (ORFs) having substantially equivalent properties, all of said genomes belonging to retroviruses which can be considered as equivalents of LAV.

It must be understood that the claims which follow are also intended to cover all equivalents of the products (glycoproteins, polypeptides, DNAs, etc..), whereby an equivalent is a product, i.e. a polypeptide which may distinguish from a determined one defined in any of said claims, say through one or several aminoacids, while still having substantially the same immunological or immunogenic properties. A similar rule of equivalency shall apply to the DNAs, it being understood that the rule of equivalency will then be tied to the rule of equivalency pertaining to the polypeptides which they encode.

It will also be understood that all the litterature referred to hereinbefore or hereinafter, and all patent applications or patents not specifically identified herein but which form counterparts of those specifically designated herein must be considered as incorporated herein by reference.

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CLAIMS

1. A purified product which contains the polypeptidic backbone of a glycoprotein having a molecular weight of about 110,000 or antigen of lower molecular weight derived from the preceding one, which purified product possesses the capacity of being recognised by serums of human origin and containing antibodies against the LAV virus.

2. The purified product of claim 1 which is the purified glycoprotein.

3. The glycoprotein of claim 2 which forms complexes with concanavaline A, said complex being dissociatable in the presence of O-methyl-α-D-mannopyranoside.

4. The glycoprotein of claim 3 or claim 4, which binds lectins.

5. The glycoprotein of any one of claims which is also sensitive to the action of endoglycosidases.

6. The purified product of any one of claims 1 to 5 which has the polypeptidic backbone of the polypeptide encoded by the nucleic acid fragment extending between nucleotide numbered 6421 and nucleotide numbered <> of figure 1.

7. The purified product of claim 1 which is a polypeptide corresponding to any of those encoded by the nucleotide sequences which extend respectively between the following positions :

a) from about 6171 to about 6276
b) " " 6336 " " 6386
c) " " 6466 " " 6516
d) " " 6561 " " 6696
e) " " 6936 " " 7006
f) " " 7611 " " 7646

which nucleotide sequences can be deduced from the LAV DNA shown in figs. 4a-4e.

8. The purified product of claim 1 which is a peptide, containing a sequence of aminoacids deducible

from the proteins encoded by the LAV DNA, which peptide is selected from the group of polypeptides defined hereafter, the numbers associated with each peptide corresponding to the positions of its N-terminal and C-terminal aminoacids starting from lysine (amino 1) coded by the AAA at positions 5734-5748 of the LAV DNA shown in figs. 4a-4e :

aminoacids 8-23 inclusive, i.e. Met-Arg-Val-Lys-Glu-Lys-Tyr-Gln-His-Leu-Trp-Arg-Trp-Gly-Trp-Lys-

aminoacids 63-78 inclusive, i.e. Ser-Asp-Ala-Lys-Ala-Tyr-Asp-Thr-Glu-Val-His-Asn-Val-Trp-Ala-Thr-

aminoacids 82-90 inclusive, i.e. Val-Pro-Thr-Asp-Pro-Asn-Pro-Gln-Glu-

aminoacids 97-123 inclusive, i.e. Thr-Glu-Asn-Phe-Asn-Met-Trp-Lys-Asn-Asp-Met-Val-Glu-Gln-Met-His-Glu-Asp-Ile-Ile-Ser-Leu-Trp-Asp-Gln-Ser-Leu-

aminocids 127-183 inclusive, i.e. Val-Lys-Leu-Thr-Pro-Leu-Cys-Val-Ser-Leu-Lys-Cys-Thr-Asp-Leu-Gly-Asn-Ala-Thr-Asn-Thr-Asn-Ser-Ser-Asn-Thr-Asn-Ser-Ser-Ser-Gly-Glu-Met-Met-Met-Glu-Lys-Gly-Glu-Ile-Lys-Asn-Cys-Ser-Phe-Asn-Ile-Ser-Thr-Ser-Ile-Arg-Gly-Lys-Val-Gln-Lys-

aminoacids 197-201 inclusive, i.e. Leu-Asp-Ile-Ile-Pro-Ile-Asp-Asn-Asp-Thr-Thr-

aminocids 239-294 inclusive, i.e. Lys-Cys-Asn-Asn-Lys-Thr-Phe-Asn-Gly-Thr-Gly-Pro-Cys-Thr-Asn-Val-Ser-Thr-Val-Gln-Cys-Thr-His-Gly-Ile-Arg-Pro-Val-Val-Ser-Thr-Gln-Leu-Leu-Leu-Asn-Gly-Ser-Leu-Ala-Glu-Glu-Glu-Val-Val-Ile-Arg-Ser-Ala-Asn-Phe-Thr-Asp-Asn-Ala-Lys-

aminocids 300-327 inclusive,i.e. Leu-Asn-Gln-Ser-Val-Glu-Ile-Asn-Cys-Thr-Arg-Pro-Asn-Asn-Asn-Thr-Arg-Lys-Ser-Ile-Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-

aminoacids 334-381 inclusive, i.e. Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg-Ala-Lys-Trp-Asn-Ala-Thr-Leu-Lys-Gln-Ile-Ala-Ser-Lys-Leu-Arg-Glu-Gln-Phe-Gly-Asn-Asn-Lys-Thr-Ile-Ile-Phe-Lys-Gln-Ser-Ser-Gly-Gly-Asp-Pro-

aminoacids 397-424 inclusive, i.e. Cys-Asn-Ser-Thr-Gln-Leu-Phe-Asn-Ser-Thr-Trp-Phe-Asn-Ser-Thr-Trp-Ser-Thr-Glu-Gly-Ser-Asn-Asn-Thr-Glu-Gly-Ser-Asp-

aminoacids 466-500 inclusive, i.e. Leu-Thr-Arg-Asp-Gly-Gly-Asn-Asn-Asn-Asn-Gly-Ser-Glu-Ile-Phe-Arg-Pro-Gly-Gly-Gly-Asp-Met-Arg-Asp-Asn-Trp-Arg-Ser-Glu-Leu-Tyr-Lys-Tyr-Lys-Val-

aminoacids 510-523 inclusive, i.e. Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-

aminoacids 551-577 inclusive, i.e. Val-Gln-Ala-Arg-Gln-Leu-Leu-Ser-Gly-Ile-Val-Gln-Gln-Gln-Asn-Asn-Leu-Leu-Arg-Ala-Ile-Glu-Ala-Gln-Gln-His-Leu-

aminoacids 594-603 inclusive, i.e. Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-

aminoacids 621-630 inclusive, i.e. Pro-Trp-Asn-Ala-Ser-Trp-Ser-Asn-Lys-Ser-

aminoacids 657-679 inclusive, i.e. Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-

aminoacids 719-758 inclusive, i.e. Arg-Val-Arg-Gln-Gly-Tyr-Ser-Pro-Leu-Ser-Phe-Gln-Thr-His-Leu-Pro-Thr-Pro-Arg-Gly-Pro-Asp-Arg-Pro-Glu-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Glu-Arg-Asp-Arg-Asp-Arg-Ser-Ile-

aminoacids 780-803 inclusive, i.e. Tyr-His-Arg-Leu-Arg-

Asp-Leu-Leu-Leu-Ile-Val-Thr-Arg-Ile-Val-
Glu-Leu-Leu-Gly-Arg-Arg-Gly-Trp-Glu-

or any combination of these peptides.

9. Peptide corresponding to the aminoacid sequences deducible from proteins encoded by LAV DNA, which peptide is selected fromthe group of polypeptides defined hereafter, the numbers associated with each peptide corresponding to the relative positions of its N-terminal and C-terminal aminoacids starting from methionine (aminoacid 1) coded by the ATG sequence at nucleotide positions 336-338 of the LAV DNA shown in figs. 4a-4e :

aminoacids 12-32 inclusive, i.e. Glu-Leu-Asp-Arg-Trp-Glu-Lys-Ile-Arg-Leu-Arg-Pro-Gly-Gly-Lys-Lys-Lys-Tyr-Lys-Leu-Lys

aminoacids 37-46 inclusive, i.e. Ala-Ser-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-

aminoacids 49-79 inclusive, i.e. Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr-Gly-Ser-Glu-Glu-Leu-Arg-Ser-Leu-Tyr-

aminoacids 88-153 inclusive, i.e. Val-His-Gln-Arg-Ile-Glu-Ile-Lys-Asp-Thr-Lys-Glu-Ala-Leu-Asp-Lys-Ile-Glu-Glu-Glu-Gln-Asn-Lys-Ser-Lys-Lys-Lys-Ala-Gln-Gln-Ala-Ala-Ala-Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Gln-Met-Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-

aminocacids 158-165 inclusive, i.e. Val-Val-Glu-Glu-Lys-Ala-Phe-Ser-

aminoacids 178-188 inclusive, i.e. Gly-Ala-Thr-Pro-Gln-Asp-Leu-Asn-Thr-Met-Leu-

aminoacids 200-220 inclusive, i.e. Met-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-

aminoacids 226-234 inclusive, i.e. Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-

aminoacids 239-264 inclusive, i.e. Thr-Thr-Ser-Thr-Leu-Gln-Glu-Gln-Ile-Gly-Trp-Met-Thr-Asn-Asn-Pro-Pro-Ile-Pro-Val-Gly-Glu-Ile-Tyr-Lys-Arg-

aminocids 288-331 inclusive, i.e. Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Ala-Glu-Gln-Ala-Ser-Gln-Glu-Val-Lys-Asn-Trp-Met-Thr-GluThr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-

aminoacids 352-361 inclusive, i.e. Gly-Val-Gly-Gly-Pro-Gly-His-Lys-Ala-Arg-

aminoacids 377-390 inclusive, i.e. Met-Met-Gln-Arg-Gly-Asn-Phe-Arg-Asn-Gln-Arg-Lys-Ile-Val-

aminoacids 399-432 inclusive, i.e. Gly-His-Ile-Ala-Arg-Asn-Cys-Arg-Ala-Pro-Arg-Lys-Lys-Gly Cys-Trp-Lys-Cys-Gly-Lys-Glu-Gly-His-Gln-Met-Lys-Asp-Cys-Thr-Glu-Arg-Gln-Ala-Asn-

aminoacids 437-484 inclusive, i.e. Ile-Trp-Pro-Ser-Tyr-Lys-Gly-Arg-Pro-Gly-Asn-Phe-Leu-Gln-Ser-Arg-Pro-Glu-Pro-Thr-Ala-Pro-Pro-Glu-Glu-Ser-Phe-Arg-Ser-Gly-Val-Glu-Thr-Thr-Thr-Pro-Ser-Gln-Lys-Gln-Glu-Pro-Ile-Asp-Lys-Glu-Leu-Tyr-

aminoacids 492-498 inclusive, i.e. Leu-Phe-Gly-Asn-Asp-Pro-Ser-

and combination of said peptides.

10. Process for obtaining the purified product of any one of claims 1 to 6, which process comprises starting from a LAV virus obtained from the supernatant of a culture of cells infected therewith and purified under conditions such that it still carries substantial proportion of envelope antigens, lysing the virus, recovering the antigens released in the supernatant and separating the purified product from other components of the lysate.

11. The process of claim 10 wherein the

starting virus has been purified by centrifugation operations, which centrifugation operations comprise particularly a first centrifugation at an angular centrifugation velocity, particularly of 10,000 rpm, enabling the removal of non-viral constituents, more particularly of cellular constituents, then a second centrifugation at higher angular velocity, particularly at 45,000 rpm, to obtain the precipitation of the virus itself.

12. A process for the production of the purified product of any of claims 1 to 9 which comprises transforming cell culture with a vector modified by a LAV DNA sequence encoding the corresponding polypeptide, which cell culture is capable of expressing said LAV DNA sequence, recovering the expression products containing the product of claims 1 to 9 of said cell culture and separating the product of claims 1 to 9 from the other expression products.

13. The process of any of claims 10, 11 or claim 12 in which the separation of the purified product is by contacting with monoclonal antibodies specifically recognizing a protein, polypeptide or glycoprotein according to any one of claims 1 to 8.

14. A method for the diagnostic of antibodies to the LAV virus in a biological fluid, particularly a human serum, which comprises contacting said biological fluid with the product of any of claims 1 to 9 under conditions suitable for enabling a complex between said antibodies and said prdouct to be formed and detecting said complex as indicative the presence of said antibodies in said biological fluid.

15. A immunogenic composition containing the purified product of any of claims 1 to 9 in association with a pharmaceutical vehicle suitable for the production of vaccines, which purified product is in an amount effective to elicit the reduction of antibodies directed against said product.

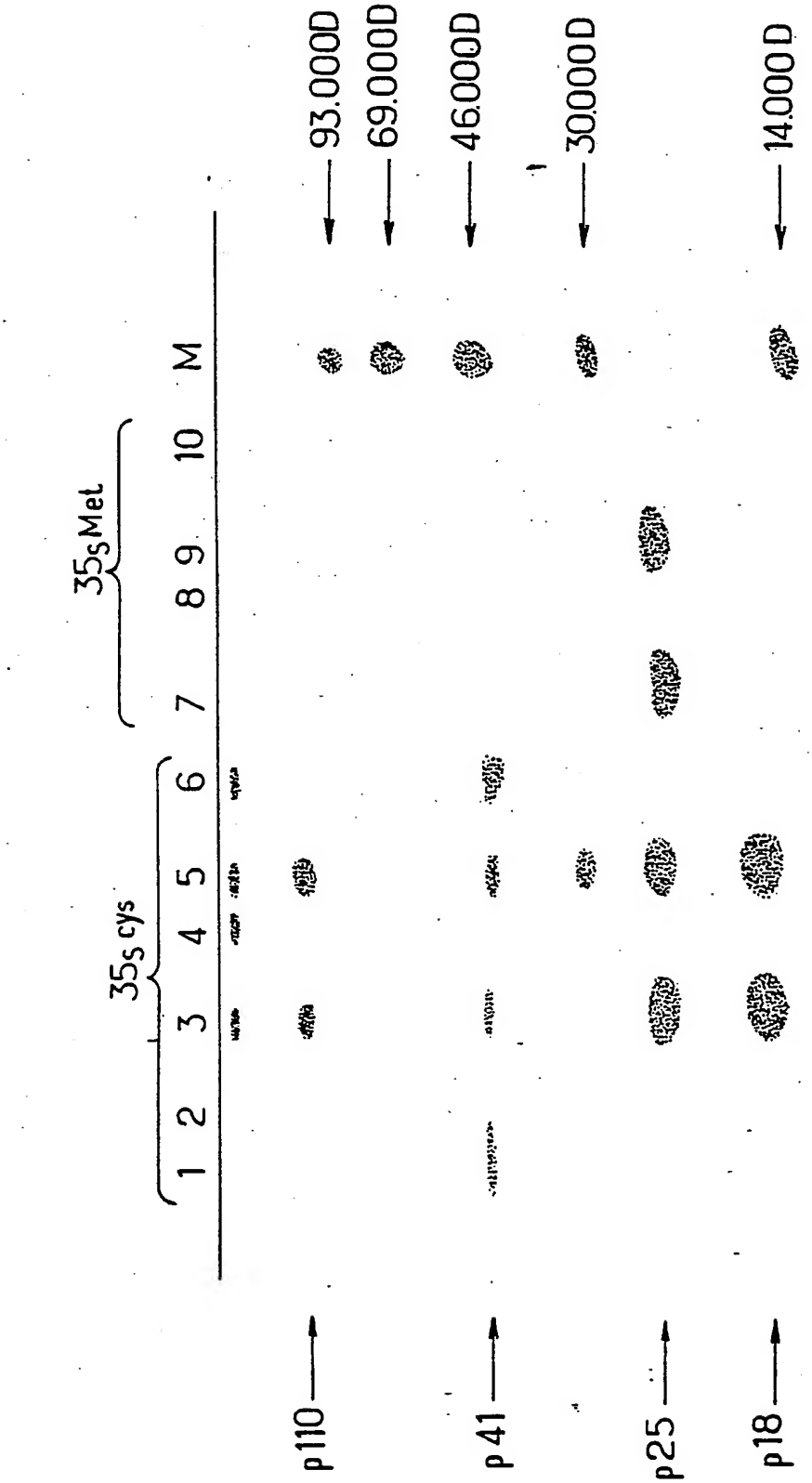
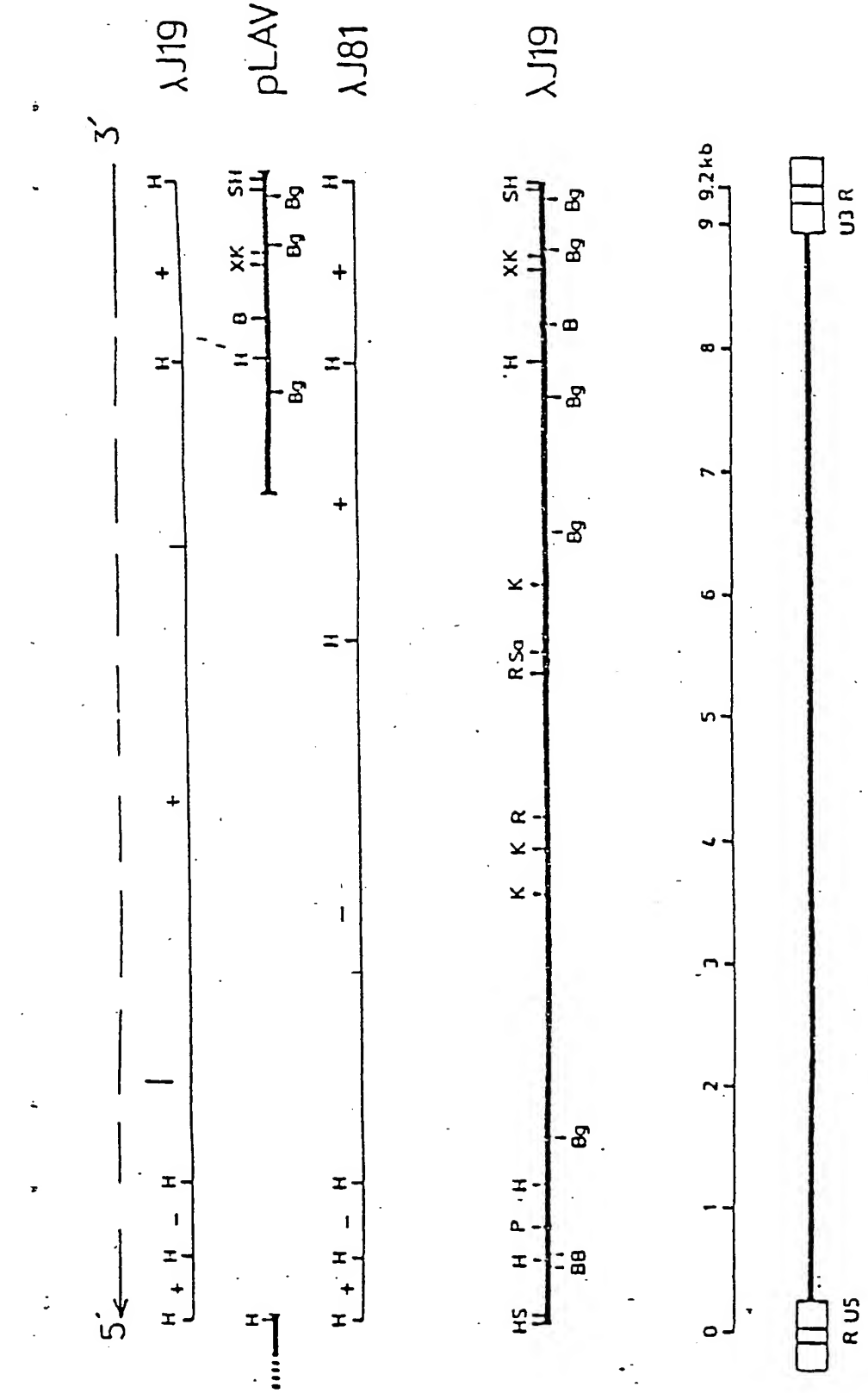

35S cys
35S Met
1 2 3 4 5 6 7 8 9 10 M
93.000D
69.000D
46.000D
30000D
14.000D
p110
p41
p25
p18

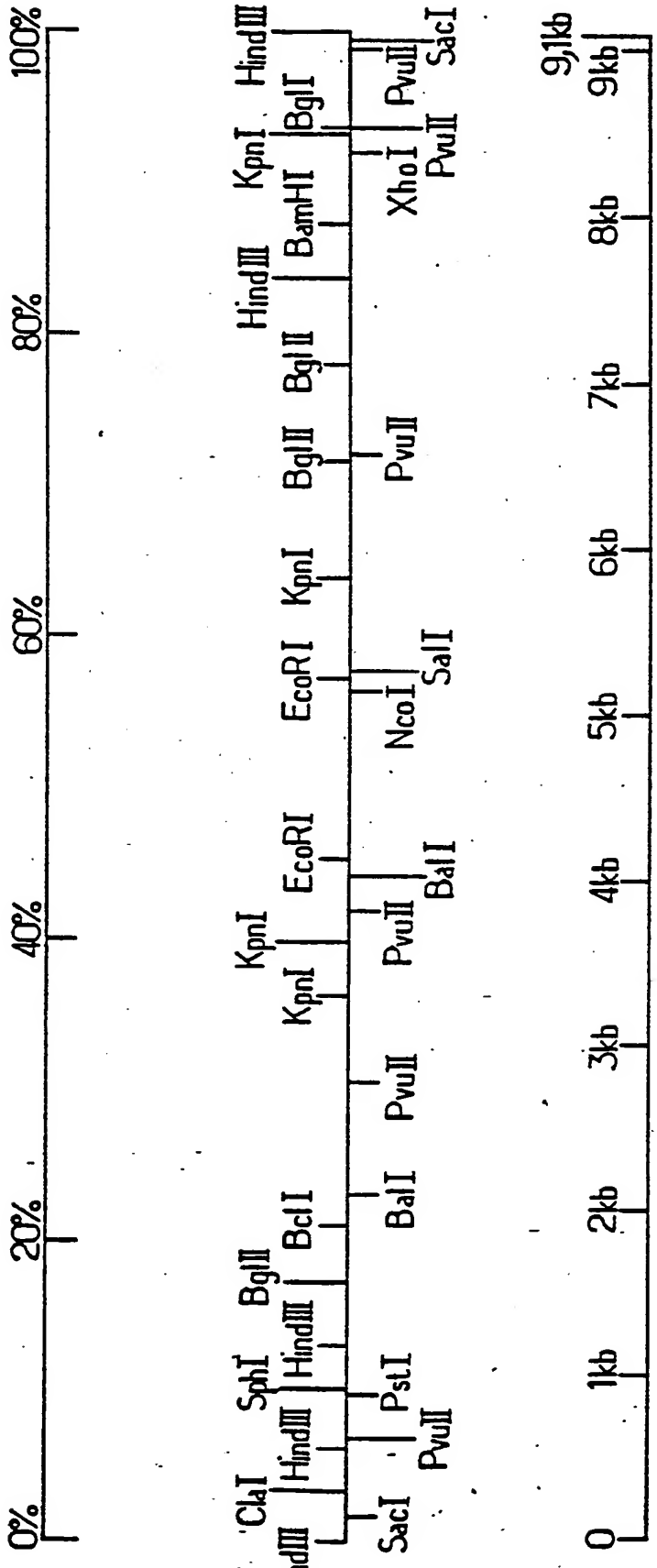
FIG.1.



H= Hind III K= Kpn I
S= Sac I R= EcoRI
B= BamHI Sa= Sal I
P= Pst I X= Xho I
Bg= Bgl II

FIG. 2

FIG. 3.


0%
20%
40%
60%
80%
100%
HindIII
ClaI
HindIII
SacI
PvuII
SphI
PstI
BglII
HindIII
BclI
BalI
KpnI
PvuII
KpnI
EcoRI
PvuII
BalI
NcoI
EcoRI
SalI
KpnI
BglII
PvuII
HindIII
BglII
BamHI
KpnI
XhoI
BglI
PvuII
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0
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5kb
6kb
7kb
8kb
9kb
9,1kb

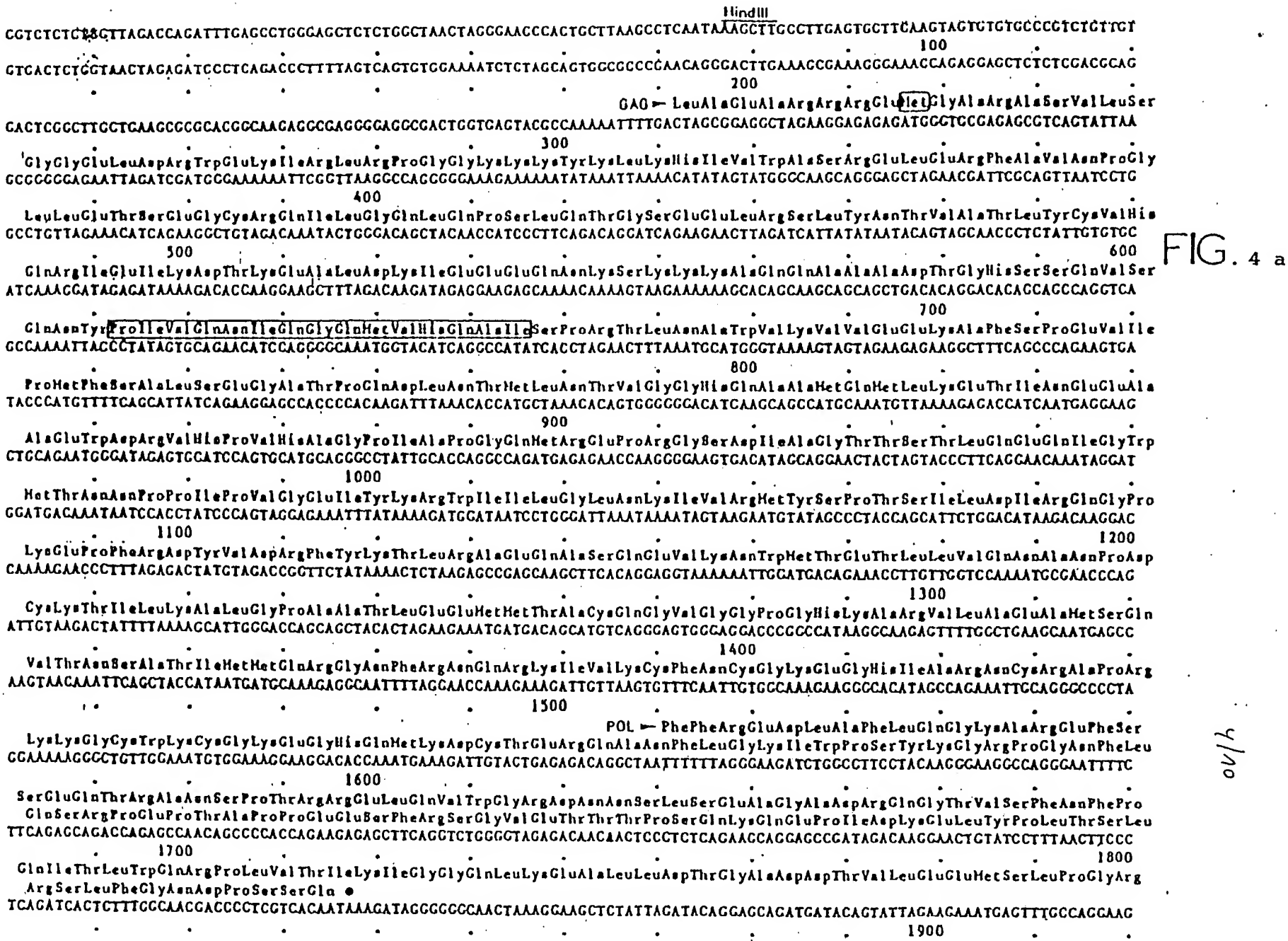
HindIII
GAG ← LeuAlaGluAlaArgArgArgGluMetGlyAlaArgAlaSerValLeuSer
POL ← PheArgGluAspLeuAlaPheLeuGlnGlyLysAlaArgGluPheSer

FIG. 4 a

FIG. 4 b

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TrpLysProLysMetIleGlyGlyIleGlyGlyPheIleLysValArgGlnTyrAspGlnIleLeuIleGluIleCysGlyHisLysAlaIleGlyThrValLeuValGlyProThrPro
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                                                                2000
ValAsnIleIleGlyArgAsnLeuLeuThrGlnIleGlyCysThrLeuAsnPheProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLys
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                                                                2100
GlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerLysIleGlyProGluAsnProTyrAsnThrProValPheAla
ACAATGGCCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTTGC
                                2200
IleLysLysLysAspSerThrLysTrpArgLysLeuValAspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLys
CATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGAATACCACATCCCGCAGGGTTAAAAAA
        2300                                                                                                    2400
LysLysSerValThrValLeuAspValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrAlaPheThrIleProSerIleAsnAsnGluThrProGlyIle
GAAAAAATCAGTAACAGTACTGGATGTGGGTGATGCATATTTTTCAGTTCCCTTAGATGAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACACCAGGGAT
                                                                2500
ArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGlySerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGln
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                                                                2600
TyrMetAspAspLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisLeuLeuArgTrpGlyLeuThrThrProAspLysLysHisGln
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                                                                2700
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GAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTAGTGGG
                                2800
LysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaLeuThrGluValIleProLeuThrGluGluAlaGluLeuGlu
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        2900                                                                                                    3000
LeuAlaGluAsnArgGluIleLeuLysGluProValHisGlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTyr
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                                                                3100
GlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgThrArgGlyAlaHisThrAsnAspValLysGlnLeuThrGluAlaValGlnLysIleThrThrGluSerIleValIle
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                                                                3200
TrpGlyLysThrProLysPheLysLeuProIleGlnLysGluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLeu
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                                                                3300
ValLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAspGlyAlaAlaSerArgGluThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGly
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                                3400
ArgGlnLysValValThrLeuThrAspThrThrAsnGlnLysThrGluLeuGlnAlaIleHisLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAspSerGlnTyrAla
AAGACAAAAAGTTGTCACCCTAACTGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAATATAGTAACAGACTCACAATATGC
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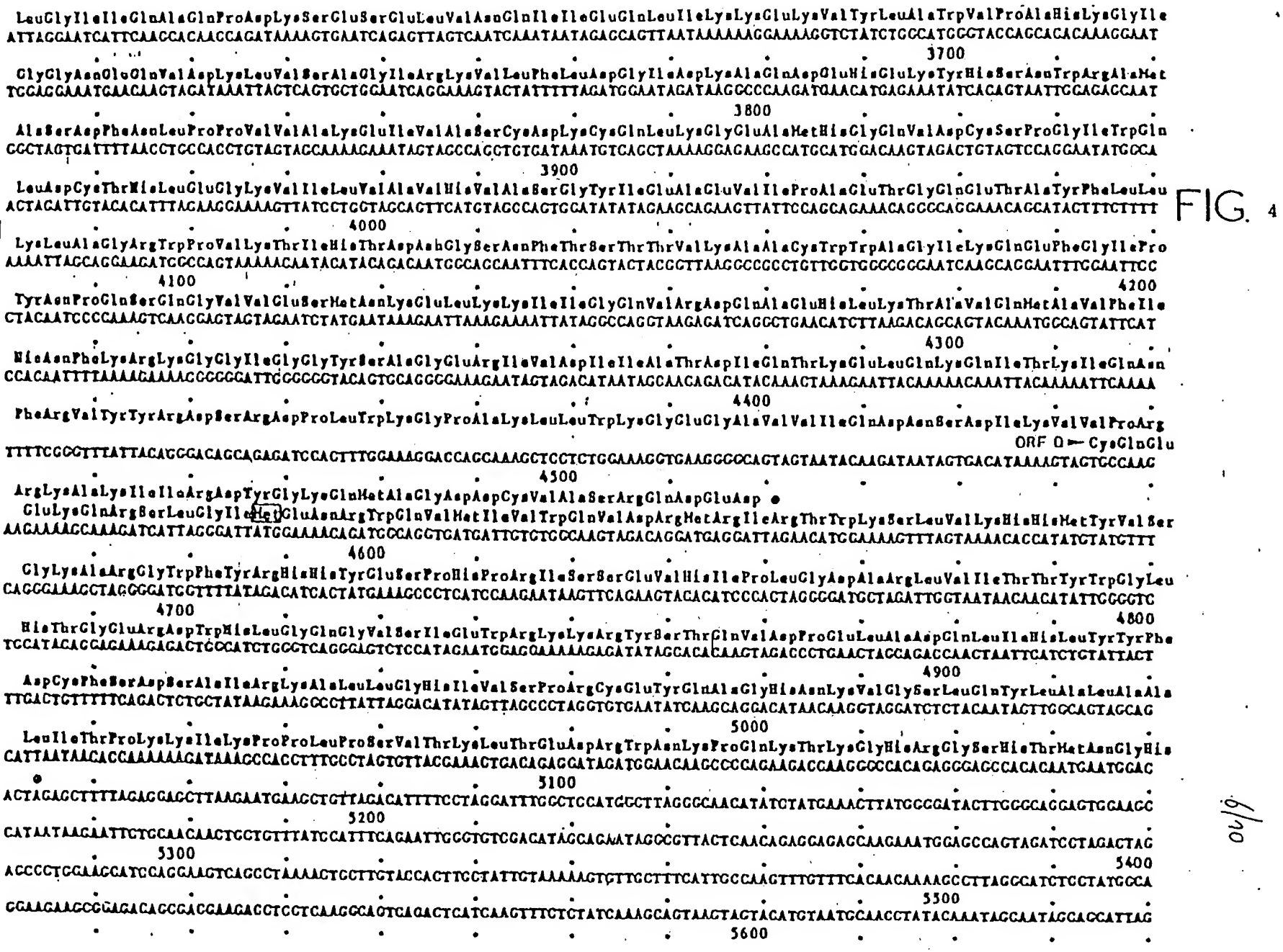
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3700
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TCGACGAAATGAACAAGTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGGCCCAAGATGAACATGAGAAATATCACAGTAATTGGAGACCAAT
3800
AlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluAlaMetHisGlyGlnValAspCysSerProGlyIleTrpGln
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3900
LeuAspCysThrHisLeuGluGlyLysValIleLeuValAlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLeu
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4000
LysLeuAlaGlyArgTrpProValLysThrIleHisThrAspAsnGlySerAsnPheThrSerThrThrValLysAlaAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIlePro
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4100
4200
TyrAsnProGluSerGlnGlyValValGluSerMetAsnLysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle
CTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTAAAGAAAATTATAGGCCAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCAT
4300
HisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleGlnThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsn
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4400
PheArgValTyrTyrArgAspSerArgAspProLeuTrpLysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProArg
ORF O→CysGlnGlu
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4500
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GluLysGlnArgSerLeuGlyIleIleGluAsnArgTrpGlnValMetIleValTrpGlnValAspArgMetArgIleArgThrTrpLysSerLeuValLysHisHisMetTyrValSer
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4600
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4800
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4900
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5000
LeuIleThrProLysLysIleLysProProLeuProSerValThrLysLeuThrGluAspArgTrpAsnLysProGlnLysThrLysGlyHisArgGlySerHisThrMetAsnGlyHis
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5100
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5200
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5300
5400
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5500
CGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGTAAGTAGTACATGTAATGCAACCTATACAAATAGCAATAGCATCATTAG
5600

FIG. 4

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ENV — LysGluGlnLysThr

TAGTAGCAATAATAATAGCAATAGTTGTGTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAAAGAAAAATAGACAGGTTAATTGATAGACTAATAGAAAGAGCAGAAGACA

5700

ValAlaMetArgValLysGluLysTyrGlnHisLeuTrpArgTrpGlyTrpLysTrpGlyThrMetLeuLeuGlyIleLeuMetIleCysSerAlaThrGluLysLeuTrpValThrVal
GTGCCAATGAGAGTGAAGGAGAAATATCAGCACTTGTGGAGATGGGGGTGGAAATGGGGCACCATGCTCCTTGGGATATTGATGATCTGTAGTGCTACAGAAAAATTGTGGGTCACAGTC

5800

TyrTyrGlyValProValTrpLysGluAlaThrThrThrLeuPheCysAlaSerAspAlaLysAlaTyrAspThrGluValHisAsnValTrpAlaThrHisAlaCysValProThrAsp
TATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGAC

5900 6000

ProAsnProGlnGluValValLeuValAsnValThrGluAsnPheAsnMetTrpLysAsnAspMetValGluGlnMetHisGluAspIleIleSerLeuTrpAspGlnSerLeuLysPro
CCCAACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCA

6100

CysValLysLeuThrProLeuCysValSerLeuLysCysThrAspLeuGlyAsnAlaThrAsnThrAsnSerSerAsnThrAsnSerSerSerGlyGluMetMetMetGluLysGlyGlu
TGTGTAAAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGATTTGGGGAATGCTACTAATACCAATAGTAGTAATACCAATAGTAGTAGCGGCGAAATGATGATGGAGAAAGGAGAG

6200

IleLysAsnCysSerPheAsnIleSerThrSerIleArgGlyLysValGlnLysGluTyrAlaPhePheTyrLysLeuAspIleIleProIleAspAsnAspThrThrSerTyrThrLeu
ATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACGTTG

6300

ThrSerCysAsnThrSerValIleThrGlnAlaCysProLysValSerPheGluProIleProIleHisTyrCysAlaProAlaGlyPheAlaIleLeuLysCysAsnAsnLysThrPhe
ACAAGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTC

6400

AsnGlyThrGlyProCysThrAsnValSerThrValGlnCysThrHisGlyIleArgProValValSerThrGlnLeuLeuLeuAsnGlySerLeuAlaGluGluGluValValIleArg
AATGGAACAGGACCATGTACAAATGTCAGCACAGTAGAATGTACACATCGAATTAGCCCAGTAGTATCAACTCAACTGCTGTTGAATGGCAGTCTAGCAGAAGAAGAGGTAGTAATTAGA

6500 6600

SerAlaAsnPheThrAspAsnAlaLysThrIleIleValGlnLeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArgLysSerIleArgIleGlnArgGlyPro
TCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGGGGACCA

6700

GlyArgAlaPheValThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCysAsnIleSerArgAlaLysTrpAsnAlaThrLeuLysGlnIleAlaSerLysLeuArgGluGlnPhe
GGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATGCCACTTTAAAACAGATAGCTAGCAAATTAAGAGAACAATTT

6800

GlyAsnAsnLysThrIleIlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSer
GGAAATAATAAAACAATAATCTTTAAGCAATCCTCAGGAGGCGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGT

6900

ThrTrpPheAsnSerThrTrpSerThrGluGlySerAsnAsnThrGluGlySerAspThrIleThrLeuProCysArgIleLysGlnPheIleAsnMetTrpGlnGluValGlyLysAla
ACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACTGAAGGAAGTGACACAATCACACTCCCATGCAGAATAAAACAAATTTATAAACATGTGGCAGGAAGTAGGAAAAGCA

7000

MetTyrAlaProProIleSerGlyGlnIleArgCysSerSerAsnIleThrGlyLeuLeuLeuThrArgAspGlyGlyAsnAsnAsnAsnGlySerGluIlePheArgProGlyGlyGly
ATGTATGCCCCTCCCATCAGCGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGACGATGGTGGTAATAACAACAATGGGTCCGAGATCTTCAGACCTGGAGGAGGA

7100 7200

AspMetArgAspAsnTrpArgSerGluLeuTyrLysTyrLysValValLysIleGluProLeuGlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArgAlaVal
GATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTG

7300

FIG. 4 a

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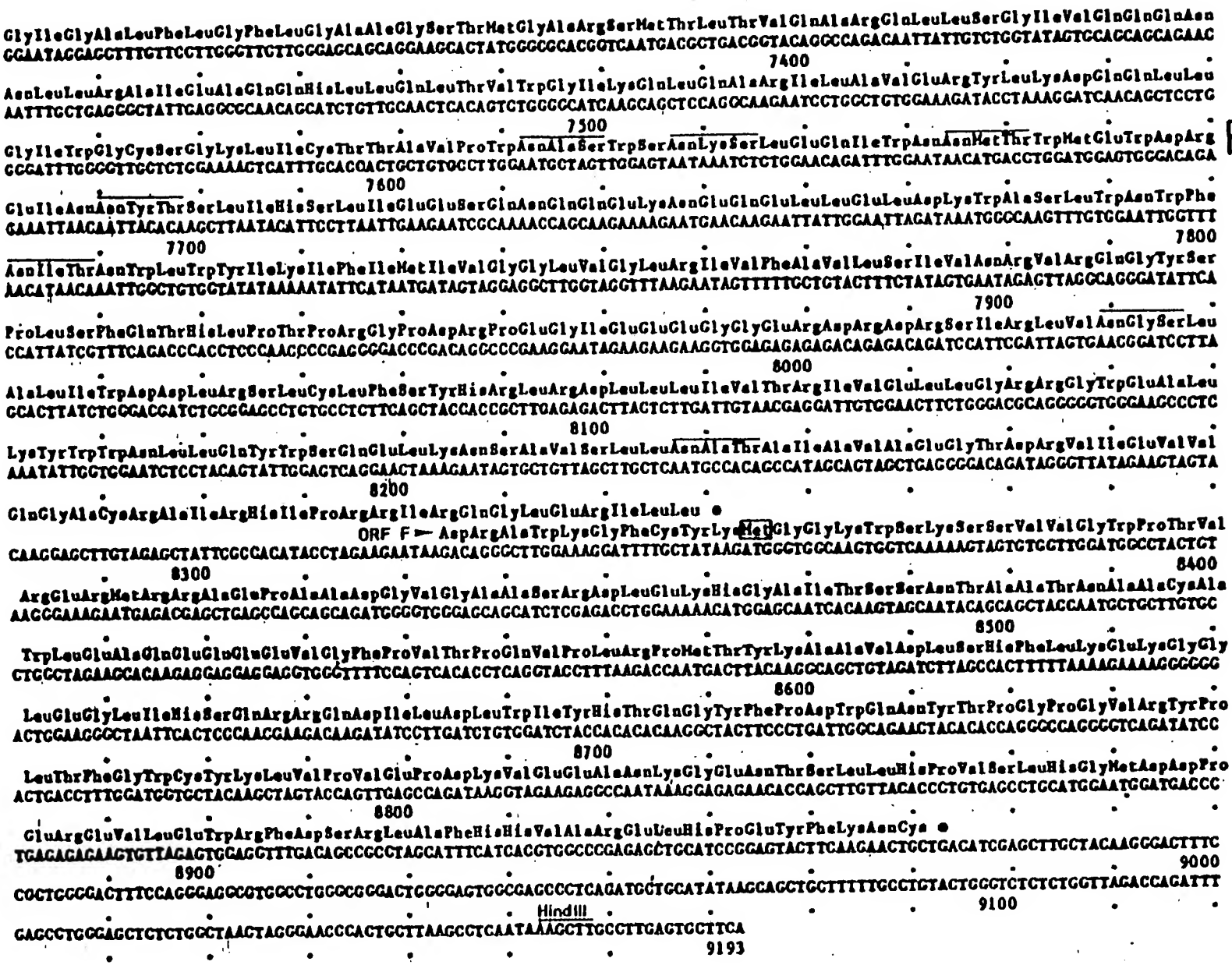
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7400
AsnLeuLeuArgAlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLysGlnLeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeuLeu
7500
GlyIleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsnAlaSerTrpSerAsnLysSerLeuGluGlnIleTrpAsnAsnMetThrTrpMetGluTrpAspArg
7600
GluIleAsnAsnTyrThrSerLeuIleHisSerLeuIleGluGluSerGlnAsnGlnGlnGluLysAsnGluGlnGluLeuLeuGluLeuAspLysTrpAlaSerLeuTrpAsnTrpPhe
7700
7800
AsnIleThrAsnTrpLeuTrpTyrIleLysIlePheIleMetIleValGlyGlyLeuValGlyLeuArgIleValPheAlaValLeuSerIleValAsnArgValArgGlnGlyTyrSer
7900
ProLeuSerPheGlnThrHisLeuProThrProArgGlyProAspArgProGluGlyIleGluGluGluGlyGlyGluArgAspArgAspArgSerIleArgLeuValAsnGlySerLeu
8000
AlaLeuIleTrpAspAspLeuArgSerLeuCysLeuPheSerTyrHisArgLeuArgAspLeuLeuLeuIleValThrArgIleValGluLeuLeuGlyArgArgGlyTrpGluAlaLeu
8100
LysTyrTrpTrpAsnLeuLeuGlnTyrTrpSerGlnGluLeuLysAsnSerAlaValSerLeuLeuAsnAlaThrAlaIleAlaValAlaGluGlyThrAspArgValIleGluValVal
8200
GlnGlyAlaCysArgAlaIleArgHisIleProArgArgIleArgGlnGlyLeuGluArgIleLeuLeu •
ORF F ⟵ AspArgAlaTrpLysGlyPheCysTyrLysMetGlyGlyLysTrpSerLysSerSerValValGlyTrpProThrVal
8300
8400
ArgGluArgMetArgArgAlaGluProAlaAlaAspGlyValGlyAlaAlaSerArgAspLeuGluLysHisGlyAlaIleThrSerSerAsnThrAlaAlaThrAsnAlaAlaCysAla
8500
TrpLeuGluAlaGlnGluGluGluGluValGlyPheProValThrProGlnValProLeuArgProMetThrTyrLysAlaAlaValAspLeuSerHisPheLeuLysGluLysGlyGly
8600
LeuGluGlyLeuIleHisSerGlnArgArgGlnAspIleLeuAspLeuTrpIleTyrHisThrGlnGlyTyrPheProAspTrpGlnAsnTyrThrProGlyProGlyValArgTyrPro
8700
LeuThrPheGlyTrpCysTyrLysLeuValProValGluProAspLysValGluGluAlaAsnLysGlyGluAsnThrSerLeuLeuHisProValSerLeuHisGlyMetAspAspPro
8800
GluArgGluValLeuGluTrpArgPheAspSerArgLeuAlaPheHisHisValAlaArgGluLeuHisProGluTyrPheLysAsnCys •
8900
9000
9100
HindIII
9193

FIG. 4

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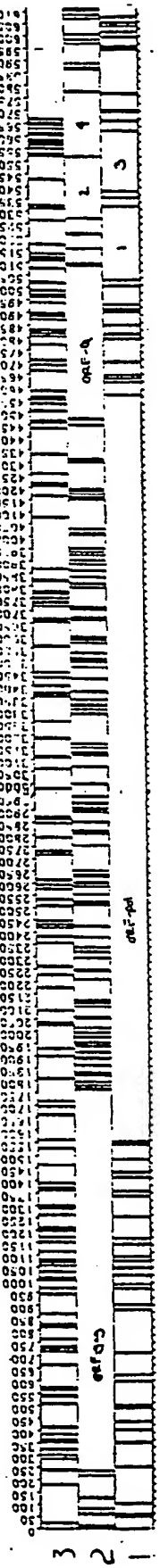

FIG. 5

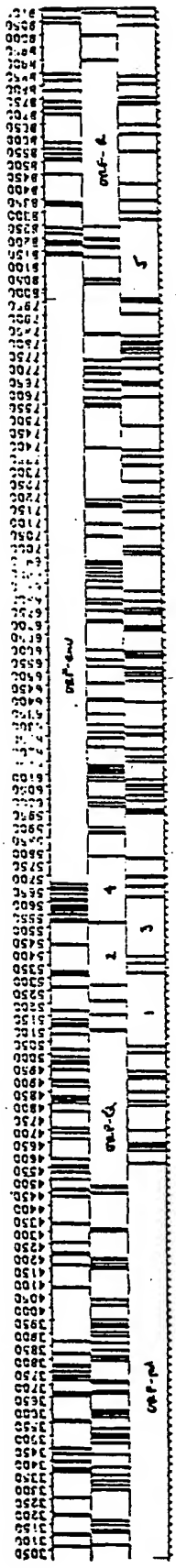

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 85/00548

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [8]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 P 21/00; C 12 N 7/02; C 12 N 15/00; G 01 N 33/569; A 61 K 39/21

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K, C 12 N, G 01 N, C 12 Q, C 12 P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Science, volume 224, 4 May 1984, (US) J. Schüpbach et al.:"Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS", pages 503-505, see page 503, right-hand column, lines 25-31,32-37; page 504, figure 2 (right), photo and figure caption, lines 19-20; page 505, left-hand column, lines 37-41 and line 63 - middle column, line 2 (cited in the application) --	1-5,14
X	Science, volume 225, 20 July 1984, (US) V.S. Kalyanaraman et al.:"Antibodies to the core protein of lymphadenopathy-associated virus (LAV) in patients with AIDS", pages 321-323, see page 323, middle column, lines 50-61 --	14
X,P	Science, volume 228, 3 May 1985, (US) W.G. Robey et al.:"Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients",	./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th February 1986	02 AVR. 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	pages 593-595, see page 593, abstract, right-hand column, lines 16-21,26-33; page 594, left-hand column, lines 1-6, 12-15 and line 19 - middle column, line 15; page 595, left-hand column, line 65 - middle column, line 5,21-26,40-45 and line 60 - right-hand column, line 3	1-5,10,14, 15
X,P	Science, volume 228, 31 May 1985, (US) J.S. Allan et al.:"Major Glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III", pages 1091-1094, see page 1091, abstract; page 1092, left-hand column, lines 54-59 and line 65 - middle column, line 3; page 1093, left-hand column, lines 19-28 and right-hand column, lines 1-14,19-24	1-5,14,15
X,P	Chemical Abstracts, volume 103, no. 19, 11 November 1985, Columbus, Ohio, (US) R. Crowl et al.:"HTLV-III env gene products synthesized in E. coli are recognized by antibodies present in the sera of AIDS patients", see page 190, abstract no. 154983e & Cell (Cambridge, Mass.) 1985, 41(3), 979-86 (Eng)	1-5,7,12, 14,15
X,P	Current Biotechnology Abstracts,0310003226, Centocor unveils rDNA AIDS assay; faces FDA, 5 August 1985, (US) Biotechnology Newswatch, volume 5, issue 15, page 3 see the whole abstract	1-5,14
P,X	Chemical Abstracts, volume 103, no. 5, 5 August 1985, Columbus, Ohio, (US) L. Montagnier et al.:"Identification and antigenicity of the major envelope glycoprotein of lymphadenopathy-associated virus", see page 263, abstract no. 34641v & Virology 1985, 144(1), 283-9	1-5,10,11, 13
Y		14
Y,P	Nature, volume 316, 4 July 1985 R.A. Weiss et al.:"Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients", pages 69-72, see page 71, right-hand column, lines 16-21	14
		./.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X,P	Science, volume 228, 31 May 1985 , (US) F. Barin et al.:"Virus envelope protein of HTLV-III represents major target antigen for antibodies in AIDS patients", pages 1094-1096, see page 1094, abstract, right-hand column, lines 16-19,21-31,60-65; page 1095, middle column, line 1 - page 1096, left-hand column, line 4; page 1096, left-hand column, lines 10-26 and line 61 - middle column, line 12	1-5,10,14
P,X	Cell, volume 40, January 1985, Cambridge, Mass. (US) S. Wain-Hobson et al.:"Nucleotide sequence of the AIDS virus, LAV", pages 9-17, see page 9, right-hand column, lines 7-10; page 13, right-hand column, line 40 - page 14, left-hand column, line 5; page 14, left-hand column, lines 14-18	1-5,14,15
X,P	Nature, volume 313, 7 February 1985 M.A. Muesing et al.:"Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus", pages 450-458, see page 455, left-hand column, lines 8-12 and line 41 - right-hand column, line 6; page 457, right-hand column, lines 12-22	1-5
X,P	Chemical Abstracts, volume 103, no. 7, 19 August 1985, Columbus, Ohio, (US) J. Schneider et al.:"A glycopolypeptide (gp 100) is the main antigen detected by HTLV-III antiserums", see page 430, abstract no. 52370k & Med. Microbiol. Immunol.1985 174(1), 35-42 (Eng)	1-5
X,P	Chemical Abstracts, volume 103, no. 15, 14 October 1985, Columbus, Ohio, (US) M.G. Sarngadharan et al.:"Immunological properties of HTLV-III antigens recognized by sera of patients with AIDS and AIDS-related complex and of asymptomatic carriers of HTLV-III infection", see page 551, abstract no. 121329t & Cancer Res. 1985, 45(9, Suppl.) 4574-7 (Eng)	1-5

./.

International Application No. PCT/EP 85/00548

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X,P	Nature, volume 312, 20/27 December 1984, London, (GB) M. Alizon et al.:"Molecular cloning of lymphadenopathy-associated virus", pages 757-760, see page 758, left-hand column, lines 28-54; page 760, left-hand column, lines 49-52 (cited in the application)	9,10,12
X,P	Nature, volume 315, 9 May 1985, London, (GB) N.T. Chang et al.:"An HTLV-III peptide produced by recombinant DNA is immuno-reactive with sera from patients with AIDS", pages 151-154, see page 151, right-hand column, lines 7-11; page 152, left-hand column, lines 2-16, right-hand column, lines 8-16; page 154, left-hand column, lines 20-24	12,14,15
X,P	WO, A, 84/04327 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 8 November 1984, see page 1, lines 1-6; page 2, line 14 - page 3, line 2; page 4, lines 4-10; page 5, lines 1-6; page 6, lines 5-9; page 9, lines 11-31	10,14
X,P	Biotechnology, volume 3, October 1985 T.W. Chang et al.:"Detection of antibodies to human T-cell lymphotropic virus-III (HTLV-III) with an immunoassay employing a recombinant escherichia coli-derived viral antigenic peptide", pages 905-909, see page 905, abstract, left-hand column, lines 20-24 and right-hand column, lines 2-5,17-24; page 907, right-hand column, line 60 - page 908, left-hand column, line 14; page 908, left-hand column, lines 36-38	12,14
X,P	US, A, 4520113 (R.C. GALLO et al.) 28 May 1985, see column 3, lines 4-9,16-21; column 6, lines 50-58; claim 1	14
A	EP, A, 0113078 (JURIDICAL FOUNDATION JAPANESE FOUNDATION FOR CANCER RESEARCH) 11 July 1984, see page 1, line 34 - page 2, line 5; page 6, line 27 - page 7, line 12	1-15
A	EP, A, 0115459 (UNIVERSITY OF MEDECINE AND DENTISTRY OF NEW JERSEY) 8 August 1984, see page 1, line 31 - page 2, line 16; claim 1	14

./.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	Nature, volume 295, no. 5845, 14 January 1982, (GB) G.R. Dreesman et al.:"Antibody to hepatitis B surface antigen after a single inoculation of uncoupled synthetic HBsAg peptides", pages 158-160, see abstract page 158, lines 20-30	15

V. [x] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [x] Claim numbers 6, because ~~they relate to subject matter not required to be searched by this Authority, namely~~:

1/ A number (of nucleotide) is lacking in claim 6, line 22, and it is not possible to get the number from the description

2/ Claim 6 refers to figure 1 for a nucleotide number, and this is impossible since figure 1 is a protein pattern after gel electrophoresis

2. [ ] Claim numbers....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/EP 85/00548 (SA 11141)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 24/03/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4520113	28/05/85	WO-A-	8504903	07/11/85
		AU-A-	4355985	15/11/85
EP-A- 0113078	11/07/84	JP-A-	59104325	16/06/84
EP-A- 0115459	08/08/84	AU-A-	2376084	02/08/84
		JP-A-	61022255	30/01/86